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Contents

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THE USE OF POLYVINYL ALCOHOL IN THE COLORIMETRIC DETERMINATION OF MAGNESIUM IN PLASMA OR SERUM BY MEANS OF TITAN YELLOW[1]

BY F. C. HEAGY[2]

Abstract

The colorimetric estimation of magnesium in plasma or serum by means of Titan Yellow requires an agent to stabilize the colored dye complex formed between magnesium hydroxide and Titan Yellow. Details are given of a method in which polyvinyl alcohol is used to maintain dispersion of the dye lake. A single estimation can be done on 2 cc. of serum.

The Titan Yellow method for estimating magnesium in plasma or serum is rapid, easy, and accurate (1). Serum proteins are precipitated by means of trichloracetic acid, Titan Yellow is added to the resulting water-clear filtrate, and on addition of sodium hydroxide a red magnesium hydroxide – Titan Yellow complex is formed that can be measured colorimetrically. In the method reported by Garner (1) gum ghatti was used to maintain the dye lake, but in our hands this substance was not consistently effective. In the following method polyvinyl alcohol (P.V.A.) is used as a stabilizing agent. Otherwise the method is similar to that of Garner with modifications for use with an Evelyn photoelectric colorimeter and a smaller initial sample.

Method

SOLUTIONS

Stock standard—dissolve 0.500 gm. of pure magnesium ribbon in distilled water containing a minimum of hydrochloric acid and dilute to one liter.

Working standard—4 cc. of the stock standard is diluted to 100 cc. (1 cc. of the working standard contains 0.02 mgm. of Mg).

Trichloracetic acid—10% (wt./vol.).

P.V.A. solution—1 : 1000; one gram of polyvinyl alcohol No. RH-349 (Canadian Industries Limited. Medium viscosity) is stirred into cold water and dissolved by gentle heating and stirring. The solution is then made up to 1000 cc. and stored for use.

[1] *Manuscript received June 2, 1948.*

Contribution from the Department of Biochemistry, University of Western Ontario, London, Ont.

[2] *Medical Research Fellow, National Research Council, Canada.*

Titan Yellow solution—0.05%; 100 mgm. dissolved in water, made to 200 cc., and filtered. The solution is stored in a brown bottle and kept out of strong light. Fresh solution is made up about every two weeks.

Sodium hydroxide—4 *N*.

Procedure

To 2 cc. of serum in a 15 cc. centrifuge tube add 4 cc. of distilled water and 2 cc. of 10% trichloracetic acid by burette. Mix well, let stand five minutes, centrifuge for five minutes at 2500 r.p.m., and filter through a Whatman No. 44 filter paper. Reserve the filtrate. To 4 cc. of the water-clear filtrate in a colorimeter tube add 4 cc. distilled water, 1 cc. P.V.A. solution, 1.5 cc. 0.05% Titan Yellow, and 2 cc. 4 *N* sodium hydroxide by burette in that order, mixing well between each step.

A blank is prepared by taking 1 cc. 10% trichloracetic acid, 7 cc. distilled water, 1 cc. P.V.A. solution, 1.5 cc. 0.05% Titan Yellow, and 2 cc. 4 *N* sodium hydroxide.

A standard is prepared by taking 1 cc. of the working standard, 1 cc. 10% trichloracetic acid, 6 cc. distilled water, 1 cc. P.V.A. solution, 1.5 cc. 0.05% Titan Yellow, and 2 cc. 4 *N* sodium hydroxide.

After five minutes the solutions are compared in an Evelyn photoelectric colorimeter with the No. 520 filter. The serum magnesium concentration is calculated by reference to a calibration curve (Fig. 1) obtained by analysis of standard magnesium solutions.

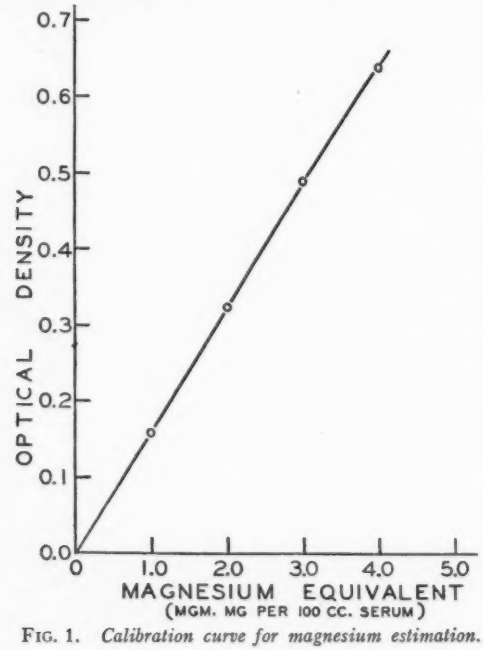

FIG. 1. *Calibration curve for magnesium estimation.*

Results

Polyvinyl alcohol consistently stabilized the dye lake. The stabilizing effects of 1 cc. of 1 : 1000 P.V.A. and 1 cc. of 1 : 1000 gum ghatti are compared in Table I. The same amount of magnesium is present in both tubes. P.V.A.

TABLE I

COMPARISON OF STABILIZING EFFECT OF POLYVINYL ALCOHOL AND GUM GHATTI

Time		Optical density	
Hr.	Min.	Polyvinyl alcohol	Gum ghatti
	3	0.367	
	5		0.149
	6	0.369	
	10		0.146
	12	0.377	
	15		0.138
	22	0.367	
	30		0.137
	37	0.367	
1	16	0.367	
1	30		0.116
2	16	0.362	
3	30		0.121
5	40	0.362	
6	—		0.116
7	—	0.362	
24	—	0.372	0.152
Mean		0.367	0.134
Standard deviation		±0.0045	±0.014
Coefficient of variation		1.2	10.4

and gum ghatti are used in the respective blanks. The coefficient of variation is 1.2 with polyvinyl alcohol and 10.4 with gum ghatti. Gum ghatti is less effective than the table indicates because shaking the tube resuspended the precipitate of magnesium hydroxide – dye complex that settled out during the longer intervals. Even after 24 to 48 hr., P.V.A.-stabilized solutions remain dispersed. It is also seen in Table I that the optical density for the same concentration of magnesium with P.V.A. is more than double that with gum ghatti. This means an increase in sensitivity and hence a smaller sample can be used. Using a spectrophotometer it was found that P.V.A. does not shift appreciably the zone of maximum absorption by the dye lake.

Garner's paper does not indicate the effect of phosphate on magnesium estimation by Titan Yellow. Table II shows that added phosphate in a concentration 100 times the normal serum phosphate concentration does not affect the estimation of magnesium.

In 13 estimations on pooled serum filtrates the calculated magnesium concentration was 2.56 ± 0.08 (S.d.) mgm. %.* In 10 estimations on

*2.56 mgm. % = 2.56 mgm. of magnesium per 100 cc. of serum.

individual filtrates of a serum the estimated magnesium concentration was 2.47 ± 0.13 (S.d.) mgm. %. Recovery experiments gave an average recovery of 99% ± 3.3% (S.d.).

TABLE II

EFFECT OF ADDED PHOSPHATE ON MAGNESIUM ESTIMATION

Phosphate added (as Na_2HPO_4), mgm. % P	Magnesium estimated, mgm. % Mg
310	2.04
31	1.99
3.1	2.13
0	1.97

Note: *Magnesium present, 2.00 mgm. %. Values calculated show concentration equivalent in serum.*

Discussion

Polyvinyl alcohol effectively stabilizes the magnesium hydroxide – dye complex formed during the estimation of magnesium by means of Titan Yellow. It is preferred to gum ghatti because it is more effective as a stabilizing agent, it is a pure chemical substance, it can be prepared by simple solution rather than by leeching, and it increases the sensitivity of the method.

Acknowledgments

I wish to thank Prof. A. B. Macallum and Dr. H. A. De Luca for advice during this work.

Reference

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STUDIES ON AMOEBIASIS IN CANADA

PART II. A METHOD FOR OBTAINING VIABLE CYSTS OF *ENTAMOEBA HISTOLYTICA* FREE FROM BACTERIA[1]

By M. J. Miller[2] and W. R. Firlotte[3]

Abstract

A method for obtaining cysts of *Entamoeba histolytica* free from bacteria is described. The method involves the centrifugation and levitation of the cysts, and their passage through a series of solutions containing penicillin and streptomycin by means of a micropipette. Thousands of sterile cysts may be obtained by this method in a few hours.

Since the pioneer work of Boeck and Drbohlav (1) on the *in vitro* culture of *Entamoeba histolytica*, this parasite has been grown on artificial media with more or less success in different parts of the world. The original Locke–egg–serum medium has been enriched, simplified, and completely changed by various workers, but the fundamental growth requirements of *E. histolytica* are still unknown. Until such time as the basic factors determining and influencing the growth of *E. histolytica* are elucidated, knowledge of the disease complex, amoebiasis, will remain inadequate.

The chief obstacle to any study on the growth requirements of *E. histolytica* has been the inability to grow the parasite outside of its host in the absence of bacteria. Jacobs (2) reported the multiplication of *E. histolytica in vitro* when dead bacteria were substituted for the living organisms. However, he was unable to obtain growth after a few transfers. Recently, Shaffer *et al.* (5, 6, 7) reported on the growth of amoebae in the absence of actively multiplying bacteria and possibly in the absence of living bacterial cells. However, their medium contained practically all the products of an actively growing bacterial culture.

The task of obtaining living amoebae free from bacterial contamination, a prerequisite to any attempt to grow this parasite in the absence of bacteria, is not an easy one. The most successful method developed to date is that used by Rees and his collaborators who employed a micropipette to pick out cysts of *E. histolytica*, and subsequently freed them from bacteria by transfers through a series of sterile solutions thus gradually eliminating the bacteria by dilution. Bactericidal chemical compounds have been used with limited success by several workers. The antibiotics, penicillin and streptomycin, were used by Shaffer *et al.* (5, 6, 7) working with trophozoites in culture, but

[1] *Manuscript received July 27, 1948.*

Contribution from the Institute of Parasitology (McGill University), Macdonald College, Que., with financial assistance from the National Research Council, awarded through the Division of Medical Research.

[2] *Associate Professor, McGill University; Research Assistant, Institute of Parasitology.*

[3] *Graduate Assistant.*

they found it necessary to replace the living bacteria by the undamaged products of a living bacterial culture to sustain growth, so the advantage was not a marked one.

The writers' experience with the use of a variety of bactericidal chemical agents in an attempt to rid cysts of *E. histolytica* from their accompanying bacteria has not been a happy one. It was found invariably that cysts of the amoebae were more susceptible to the chemical agent than were at least one or more species of the accompanying bacteria. The method of elimination by dilution of the bacteria accompanying cysts of *E. histolytica* by means of a micropipette as described by Rees (4) has been tested but was found very exacting and time-consuming. At present, successful use is being made of a method that involves the use of centrifugation, flotation of the cysts, immersion of the cysts in penicillin and streptomycin, and finally, dilution of the cysts in sterile solution with the aid of a micropipette. This method, which sounds complicated, is actually carried out with a minimum of effort provided a micropipette is available. Cysts of *E. histolytica* passed in stools are used in preference to cysts obtained in culture tubes mainly because the cysts in the latter are not uniformly quadrinucleate, nor are *in vitro* encystation techniques consistently successful. The method used is as follows:—

(1) The stool is broken up, suspended in tap water and sieved through a fine mesh screen (80 meshes per linear inch).

(2) The suspension of faeces and cysts in water is washed in tubes, 2.5 by 5.0 cm., for five minutes at 1500 r.p.m.; this is repeated until the supernatant fluid is clear (five to seven times).

(3) The washed sediment from all the tubes is pooled, and approximately 1 ml. amounts are placed in sterile Wassermann tubes, and sterile Ringer's solution added to nearly fill the tubes. The tubes are centrifuged for three minutes at 1500 r.p.m., and the supernatant fluid discarded.

(4) About 2 cc. of zinc sulphate solution, sp. gr. 1.100, is added to the sediment of each tube, the sediment is carefully broken up in the solution, and additional zinc sulphate solution added to within 5 mm. of the top of the tube. This is centrifuged for 90 sec. at 1500 r.p.m. The cysts, which are now floating on the surface of the zinc sulphate solution in the tube, are picked up by means of a wire loop 5 mm. in diameter and washed off into a Wassermann tube containing sterile Ringer's solution. Usually, 10 loopfuls will collect the large majority of the cysts.

(5) After the cysts from 20 or more flotations have been collected and washed off into the tube of sterile Ringer's solution, this tube is centrifuged for four minutes at 1500 r.p.m. The supernatant fluid is carefully pipetted off to within 1 cm. of the bottom, sterile Ringer's solution added, and the centrifugation repeated as above. Again the supernatant fluid is pipetted off to within 1 cm. of the bottom.

(6) The remaining fluid and cysts are transferred to a small Petri dish (10 by 60 mm.) containing 8 cu. mm. of sterile Ringer's solution to which has been added penicillin to a concentration of 500 units per ml., and streptomycin to a concentration of 1000 units per ml.

(7) The cysts, which are now free from practically all faecal debris and bacteria,* are picked up by means of the micropipette and transferred to a new Petri dish containing the antibiotics in sterile Ringer's solution. This is repeated five times although the cysts are usually sterile following the second transfer.

The micropipette has been built on the same principle as that described by Rees (4) and is used in the same manner. It is essentially a microscope frame with a mechanical stage attached. The pipette, which is made by drawing out glass tubing, is controlled by a mercury column operated through a thumb screw attached to a tuberculin syringe (Figs. 1, 2, and 3). The pipette is sterilized by redrawing as described by Rees (4). The writers have modified the basic design so that the entire pipette mechanism is mounted on the mechanical stage and moves as a unit when the pipette is moved; the danger of breaking the glass tube connecting the mercury from the syringe to the pipette is thus obviated.

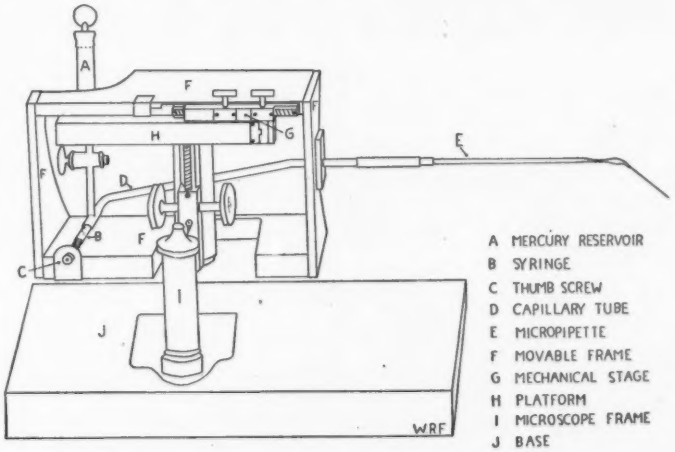

FIG. 1. *Diagram of micropipette to show the working parts in detail.*

Stools that show less than one *E. histolytica* cyst per low power field on simple smear will not give a heavy yield by this method. However, when necessary, the writers have used stools showing considerably less than one cyst per low power microscope field and were always sure of obtaining several

** The examination of faecal debris and cysts at this stage for bacteria by culture methods has always revealed the presence of a species of Streptococcus, the only species apparently resistant to the antibiotics used.*

hundred sterile cysts. On the other hand, if a stool shows a heavy infection, with five or more cysts per low power field, thousands of sterile cysts can be obtained. In all cases, the entire procedure should not require more than eight hours' working time. In mixed infections some difficulty may be experienced in separating *E. histolytica* cysts from those of other species. However, *Entamoeba coli* and *Endolimax nana*, which are the usual contaminants, can be differentiated in most cases by size alone.

As shown previously by one of us (3), institutions such as orphanages, etc., show a high incidence of *E. histolytica* infections in Canada, and this probably holds true for other parts of the world. Cysts used in these experiments were obtained from stools collected in a boys' orphanage. The well known irregularity with which *E. histolytica* cysts appear in the stool in most infections makes it necessary in many cases to obtain several consecutive stools from the same cases before a satisfactory stool is found.

The writers have used this method successfully for initiating cultures of *E. histolytica* growing with single strains of bacteria and are now studying the cultural characteristics of the amoebae growing in this manner.

To initiate cultures with sterile cysts, from 10 to several hundred cysts have been inoculated. Routinely, six tubes are inoculated: two with 10 cysts each, two with 25 cysts each, and two with 100 cysts each. The bacteria to be tested are added immediately after the cysts have been inoculated into the culture tubes. To date, 10 species of bacteria have been tested, the majority of which have sustained growth of amoebae in a modified Boeck and Drbohlav (1) culture medium. The results of these experiments will be the subject of a later report.

Acknowledgments

The photographs were made by Mr. J. B. Poole to whom the authors express their thanks.

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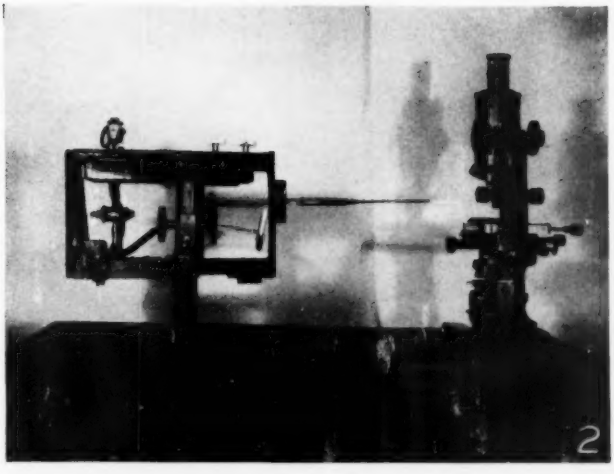
PLATE I



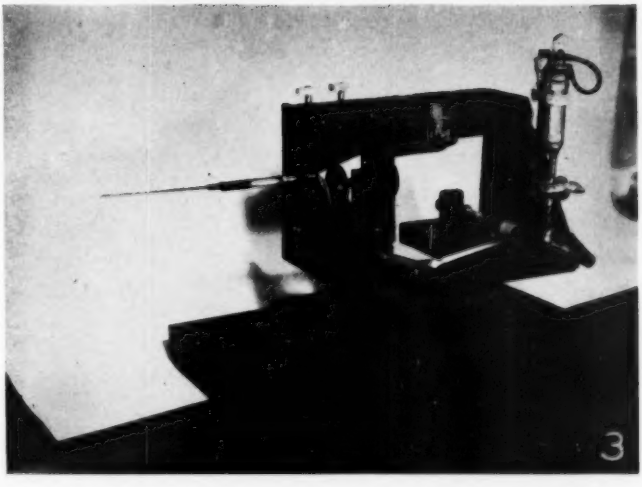


FIG. 2. *Micropipette set up for collecting cysts of Entamoeba histolytica.*

FIG. 3. *Micropipette viewed from behind.*

SOME EFFECTS OF THE ADMINISTRATION OF THORIUM NITRATE TO MICE[1]

By S. J. Patrick[2]

With the Technical Assistance of Emma Marie Cross

Abstract

A method for the determination of microgram amounts of thorium in tissues by the use of the thorium isotope UX_1 was developed.

When an aqueous solution of thorium nitrate was administered orally to mice, the LD_{50} was found to be between 1.76 and 2.0 gm. of thorium per kgm. body weight. Mice supplied with a 0.1% solution of thorium nitrate as their only source of water exhibited a greater mortality than was found in a control group. The subcutaneous injection of 50 mgm. of thorium as a thorium nitrate solution into mice produced an acute, necrotizing, inflammatory reaction at the site of injection, but no other effects. The exposure of a group of mice to an aerosol of thorium nitrate solution on each of 90 days for 40 min. each day had no effect on their mortality. The average initial concentration of thorium for each exposure to the aerosol of thorium nitrate was 330 mgm. thorium per cu. m. of air and the average final concentration was 114 mgm. of thorium per cu. m. of air.

Evidence was obtained that all the thorium administered orally as thorium nitrate solution to mice is excreted by way of the feces and that no absorption from the gastrointestinal tract takes place. It is suggested that thorium nitrate owes its oral toxicity to its local action on the mucosa of the gastrointestinal tract.

No evidence was obtained that thorium was transported to the viscera and very little thorium was excreted after the subcutaneous injection of 0.5 mgm. of thorium as thorium nitrate solution into mice. Most of the thorium remained at the site of injection for at least seven days. The cutaneous application of 0.3 ml. of BAL (2,3-dimercaptopropanol) appeared to cause a slight increase in the excretion of thorium, but the distribution of thorium in the mouse was not affected by this treatment.

A group of mice was exposed for 180 min. to an aerosol of thorium nitrate that gave a maximum concentration of 107 mgm. of thorium per cu. m. of air and a final concentration of 3 mgm. of thorium per cu. m. of air. After this treatment the average amount of thorium found in and on one mouse was 256 μgm. Most of this was on the skin and most was excreted by way of the feces within a few days. Less than 0.2 μgm. of thorium was found in the liver, kidneys, spleen, blood, or femurs, immediately after the exposure or one day later. The average amount of thorium found in the lungs of one mouse immediately after the exposure was 3.12 μgm. and this amount decreased slowly over a period of 35 days.

Introduction

While a great deal of work has been done on the effects of colloidal thorium dioxide on men and animals, the literature contains few references to the metabolism of soluble thorium salts. The following investigation of the toxicity of thorium nitrate and the distribution and excretion of thorium after the administration of thorium nitrate to mice was therefore undertaken.

The Determination of Microgram Amounts of Thorium

An examination of the literature revealed few methods suitable for the determination of microgram amounts of thorium in tissues. Kuznetsov (2)

[1] *Manuscript received July 22, 1948.*
Contribution from Defence Research Chemical Laboratories, Ottawa, Canada.

[2] *Head of the Physiology Section.*

prepared the dye 2-hydroxy-3,6-disulphonaphthaleneazobenzene-2-arsonic acid, which gives a red color in the presence of thorium under acid conditions. A colorimetric method for the determination of 5 to 100 μgm. of thorium in 5 ml. of water was developed by Thomason, Perry, and Byerly (3) using this dye. In the present investigation the synthesis of the dye was accomplished through the following steps. *o*-Nitrophenylarsonic acid was prepared from *o*-nitroaniline and *o*-arsanilic acid was prepared from the *o*-nitrophenylarsonic acid by the method of Jacobs, Heidelberger, and Rolf (1). The dye was prepared by coupling the *o*-arsanilic acid with commercial "R" acid (2-naphthol-3,6-disulphonic acid) in the following manner. Two grams of arsanilic acid were dissolved in 5.0 ml. of 5 *N* hydrochloric acid and 1.0 gm. of sodium nitrite was dissolved in 4.0 ml. of water. Both solutions were cooled to 0° C. and were then combined. The resulting solution was neutralized by the dropwise addition of concentrated sodium hydroxide solution. A solution of 2.8 gm. of "R" acid in 20 ml. of 0.5% sodium hydroxide was added. The resultant dye was precipitated with two volumes of acetone, filtered, and dried *in vacuo*.

The determination of thorium by means of the dye was carried out by adding 1.0 ml. of a 0.1% solution of the dye in water to 0.2 ml. of concentrated hydrochloric acid in a 10 ml. volumetric flask, adding the solution containing the thorium and making up to volume with water. The color of the solution was compared with the color of a blank solution containing no thorium by means of a Klett photoelectric colorimeter using the 54 green filter. The amount of thorium in the solution was then determined by reference to a standard curve showing the relation between the colorimeter reading and amount of thorium in solution. It was found that the use of 1.0 ml. of the 0.1% dye solution and 0.2 ml. of concentrated hydrochloric acid permitted the convenient determination of 5 to 80 μgm. of thorium in 10 ml. of solution. Smaller amounts of dye decreased the range of the determination and strongly acid conditions resulted in a diminution of the color change. It was found that milligram amounts of sodium nitrate or nitrite, calcium nitrate, magnesium nitrate, barium nitrate, ammonium chloride, or potassium chloride did not interfere with the development of the color due to the reaction of 40 μgm. of thorium with 1.0 ml. of the dye solution. However, a few milligrams of sodium sulphate or phosphate decreased the color change.

Difficulty was encountered in the determination of thorium in tissue digests with this method. Substances present in the digests prevented the development of the color of the thorium complex. Therefore, this method was used for the determination of thorium only when no other materials were present in solution.

Other methods based on the precipitation of thorium with oxalic acid, "oxine", or other reagents are suitable only for larger quantities of thorium than were expected to be present in the material under investigation.

Thorium can be estimated by means of its radioactivity, but it was found that not less than 1 mgm. of thorium could be determined easily by counting the β particles with a Geiger-Mueller counter. However, the thorium isotope UX_1 can be easily prepared from uranium salts. This is an active β emitter, having a half-life of 24.6 days, and so can be used to trace very small amounts of thorium. In the following experiments the thorium compounds administered to the mice were enriched with the corresponding UX_1 compound and it was found a simple matter to determine the distribution of the thorium in the animal by determining the β activity of each tissue.

Table I shows the first part of the uranium series of radioactive changes and the relationship of UX_1 to its parent and daughter isotopes.

TABLE I

THE TRANSFORMATION OF URANIUM 238 BY RADIOACTIVE DECAY

Substance	Isotope	At. wt.	At. no.	Half-life	Type of disintegration	Absorption coeff. in Al
Uranium	U	238	92	4.7×10^9y.	α	—
↓						
UX_1	Th	234	90	24.6 d.	β	463 cm.
↓						
UX_2	Pa	234	91	1.15 m.	β	14.4 cm.
↓						
U II	U	234	92	2×10^6 y.	α	—
↓						
Io	Th	230	90	6.9×10^4y.	α	—

Since U II gives rise to a series of radioactive elements, none of which are uranium isotopes, and since U II and Io have extremely long half-lives, only the substances shown in Table I are present in measurable quantities in any freshly prepared uranium salt. Since UX_2 has such a short half-life, any sample of UX_1 is in equilibrium with a definite amount of UX_2 within a very short time after its isolation, but no other isotopes that emit β particles are present. A Geiger-Mueller counter of the "bell" type with an aluminum window 0.008 cm. thick was used to count the β particles. Under these conditions no α particles incident on the window can pass through, only a very small proportion of the β particles of UX_1 and about 89% of the β particles of UX_2 can pass through. Thus, the amount of UX_1 in any sample of material was determined by counting only the β particles emitted from the UX_2.

It was desirable to exclude the β particles of UX_1 from the counting because these are of low energy and so would be partially absorbed by substances in the tissue digests. The percentage absorption of the β particles of UX_1 would depend on the amount of digest present and thus the measured radioactivity of any tissue digest would depend not only on the amount of UX_1 present,

but also on the amount of inert material. The β particles of UX_2 are of high energy and it was shown that they were not appreciably absorbed by the amounts of tissue digests encountered in the experiments that were carried out.

UX_1 was separated from uranyl nitrate in the following manner. One hundred grams to 1 kgm. of uranyl nitrate hexahydrate was dissolved in peroxide free ether in a separatory funnel using 100 ml. of ether for every 100 gm. of uranyl nitrate hexahydrate. Two phases separated owing to the water of crystallization of the uranyl nitrate. The aqueous phase was found by β activity determination to contain practically all of the UX_1 and the ethereal phase contained most of the uranium. The aqueous phase was separated and made up in volume until it contained 5 gm. of uranyl nitrate per 100 ml. Ten mgm. of thorium as thorium nitrate was dissolved in the solution to serve as carrier for the UX_1. The solution was made 5 *N* with respect to hydrofluoric acid by addition of the concentrated acid and the solution was allowed to stand overnight. The precipitate of thorium fluoride was separated by centrifuging. It was found that most of the UX_1 was present in the precipitate. The precipitate was dissolved by heating with concentrated nitric acid and the solution was evaporated to dryness on the hot plate. The residue was dissolved in water, the thorium was reprecipitated with hydrofluoric acid, the precipitate was redissolved in nitric acid, and the nitric acid was boiled off. Thorium nitrate was added to the residue in sufficient quantity to give the desired specific radioactivity to the thorium. A solution of this material in water was used for the experiments on the distribution and excretion of thorium in mice.

The following method was used to determine the amount of enriched thorium in the tissues of the experimental animals. About 0.5 gm. of tissue was excised, weighed, and digested in a 30 ml. beaker with nitric and perchloric acid. The digestion was continued until the solution was practically colorless. The digest was boiled down until it was about 1 ml. in volume, and was transferred to a small Pyrex dish of internal diameter 2.5 cm. and with a wall 0.8 cm. high. The digest was evaporated to dryness with an infrared lamp. The beaker was washed with five successive portions of nitric acid, each of which was evaporated to dryness in the dish. The residue was thereby deposited in a suitably even layer over the bottom of the dish, and the weight of the residue was determined.

Dishes containing standard amounts of UX_1 were prepared and the amount of UX_1 in the tissue digest was determined by comparing the radioactivity of the digest in the dish with the radioactivity of the standard. The amount of thorium in the tissue digest was then calculated from the ratio of UX_1 to thorium in the material administered to the experimental animal.

Thorium in aqueous solutions or in urine was determined merely by pipetting an aliquot into a dish, evaporating the material to dryness, and determining its radioactivity.

Determinations of radioactivity were carried out in the following order: (1) a determination of the background counting rate, (2) a determination of the radioactivity of the standard sample of UX_1, (3) determinations of the radioactivity of the samples, (4) a redetermination of the radioactivity of the standard sample of UX_1. After use, each dish was cleaned by scrubbing with cleanser and water. The empty dish was then tested for radioactivity to make sure that no UX_1 was adhering to it.

Table II is a summary of experiments that were carried out in order to determine whether the amount or kind of inactive material in the dish had any effect on the measurement of UX_1. In these experiments, known amounts

TABLE II

THE DETERMINATION OF UX_1 IN THE PRESENCE OF MOUSE TISSUES

Tissue	No. of determinations	Amt. of tissue	Th in sample, μgm.	Method of determination	Average wt. of ash, mgm.	Average % recovery
Urine	4	0.5 ml.	20	Evaporation to dryness on dish	28	100
Urine	4	1.0 ml.	20	"	63	99
Urine	2	1.5 ml.	20	"	80	96
Urine	2	2.0 ml.	20	"	140	95
Blood	2	0.13 ml.	20	"	27	98
Blood	2	0.25 ml.	20	"	48	95
Blood	2	0.50 ml.	20	"	95	92
Liver	2	0.40 gm.	20	HNO_3, $HClO_4$ digestion	24	99
Liver	2	0.60 gm.	20	"	46	99
Intestine	2	0.40 gm.	20	"	27	99
Intestine	2	0.60 gm.	20	"	38	98
Kidney	2	0.40 gm.	40	"	25	97
Muscle	2	0.50 gm.	40	"	32	101
Skin	2	0.50 gm.	40	"	28	98
Bone (femurs)	2	0.10 gm.	40	"	87	99
Feces	2	0.10 gm.	20	"	55	100
Feces	2	0.20 gm.	20	"	120	94

of thorium enriched with known amounts of UX_1 were added to portions of mouse tissues, the UX_1 was determined, and the recovery of UX_1 was calculated. In subsequent experiments an amount of tissue was used such that the amount of residue in the dish was always less than 50 mgm. The data of Table II show that almost quantitative recoveries of UX_1 in the presence of tissues are obtained under these conditions.

The experiments shown in Table II were each carried out with an amount of UX_1 that gave about 1000 counts per minute under the conditions of counting that were used. The background counting rate was about 30 counts per minute and each sample was counted for 10 min. It is calculated that the odds are 20 : 1 that the error due to the random nature of the emission of β particles is less than $\pm$ 2%. Sometimes errors greater than this were

encountered in the determination of duplicate samples. These are attributed to factors such as unevenness in the disposition of the UX_1 and inert material in the dish and variation in counting efficiency. The sensitivity and accuracy of the determination of thorium was increased, when necessary, by increasing the ratio of UX_1 to thorium 232 in the material used.

The Toxicity of Thorium Nitrate for Mice

The acute toxicity of thorium nitrate after oral administration to mice was determined in the following manner. Groups of female albino mice were selected in such a manner that the greatest deviation of the weight of any mouse from the mean of the weight of all the mice in the group was one gram. Solutions of thorium nitrate in water containing approximately 100 mgm. of thorium per ml. were prepared. The exact concentration of thorium of each solution was determined by precipitating thorium oxalate from an aliquot part of the solution, igniting the thorium oxalate, and weighing the thorium oxide. A definite volume of the solution was administered to each mouse by means of a stomach tube consisting of a glass capillary tube, with an external diameter of about 1 mm., that was attached to a syringe by means of a short rubber tube. The mice were observed for two weeks following dosing. Pathological examinations were carried out on some of the mice that died. The results of these experiments are summarized in Table III.

TABLE III

THE TOXICITY OF THORIUM NITRATE AFTER ORAL ADMINISTRATION TO MICE

Av. wt. of mice in group, gm.	Conc. of solution, mgm. Th/ml.	Vol. of solution administered, ml.	Dose, gm. Th/kgm. body wt.	No. in group	No. dead in 14 days	Mortality, %
25	100.0	0.19	0.76	20	0	0
23	100.0	0.23	1.00	10	1	20
25	100.0	0.25	1.00	10	3	
20	100.0	0.25	1.25	20	6	30
29	101.1	0.43	1.50	10	1	35
29	98.8	0.44	1.50	10	6	
21	100.0	0.37	1.76	20	5	25
23	100.0	0.46	2.00	10	3	55
24	100.0	0.48	2.00	10	8	
25	100.7	0.56	2.25	20	18	90
23	100.7	0.57	2.50	20	20	100

A 0.8 ml. dose of a 10% solution of sodium chloride was administered to each of a group of 10 mice in order to discover any effects that might result from the administration of a comparatively large volume of a hypertonic solution. No effects were observed. Similarly no effects were observed after the administration of 0.8 ml. of a 10% solution of sodium nitrate to mice. It is concluded that the LD_{50} of thorium nitrate after oral administration to

mice is between 1.76 and 2.0 gm. of thorium per kgm. body weight and that the toxicity is due to the thorium ion. Post-mortem examination showed occasional intestinal haemorrhage, but no damage to other internal organs.

An experiment was carried out to discover any effects of the continued ingestion of thorium nitrate. In this experiment a group of 20 mice weighing between 24 and 26 gm. were supplied with 0.1% thorium nitrate solution as their only source of water. It was found that this group of mice drank a volume of thorium nitrate solution comparable to the volume of water drunk by a control group of mice in the same length of time. After a month no changes were noted in the animals receiving the thorium nitrate. However, after four months 50% of the mice receiving the thorium nitrate were dead, whereas only 10% of the control mice were dead. Although no lesions that could be ascribed to the action of thorium nitrate specifically were noted in the animals that died, this experiment suggests that continued ingestion of thorium nitrate has a deleterious effect.

The subcutaneous injection of 50 mgm. of thorium as thorium nitrate in 0.5 ml. of water into mice produced an acute, necrotizing, inflammatory reaction at the site of injection, but no lesions were noted in the viscera following this treatment. None of the five mice treated in this manner died within 14 days after treatment.

An experiment was carried out to discover any effects of repeated exposures of mice to an aerosol of a thorium nitrate solution. A group of 20 mice were exposed to the thorium nitrate aerosol on each of 90 week days for 40 min. each day. Each exposure was carried out in the following manner. Three ml. of a 10% solution of thorium nitrate in water were sprayed into a chamber of 125 liter capacity by means of a small glass atomizer using an air pressure of 18 lb. per sq. in. The mice were then placed in the chamber in a wire cage with an individual compartment for each mouse. The concentration of thor um in the air of the chamber was determined by passing 2.0 liters of the air through a gas washing bottle containing 25 ml. of water and determining the thorium content of the water. The thorium content of the air in the chamber was determined again just before the mice were removed from the chamber 40 min. after they were put in. The average initial concentration of thorium in the air in the chamber was 330 mgm. of thorium per cu. m. of air, and the average final concentration was 114 mgm. of thorium per cu. m. The mortality of these mice was not significantly different to that of a control group of mice.

The Distribution and Excretion of Thorium After the Administration of Thorium Nitrate to Mice

The following general methods were used in the experiments on the distribution and excretion of thorium after the administration of thorium nitrate to mice. Female albino mice were used in all the experiments. Aqueous solutions of thorium nitrate enriched with UX_1 nitrate were prepared and

the thorium content and radioactivity of each solution were determined by the methods that have been described. The activity of the UX_1 was such that one mouse was never exposed to more than about one μc.

After administration of the thorium nitrate either orally, subcutaneously, or by inhalation, a group of the treated mice were placed in a metabolism cage from which urine and feces could be collected and which was supplied with a water bottle. The mice were fed twice a day for one hour intervals in a separate cage. The urine and feces excreted during feeding periods were recovered from the bottom of the feeding cage. The feces were collected each day and dissolved by heating with nitric acid. Aliquot parts of this solution were analyzed for thorium. The urine was collected each day, centrifuged, and the supernatant urine and residue were analyzed separately.

Groups of mice were sacrificed at various intervals after the administration of the thorium nitrate. At this time each mouse was anesthetized, 0.05 ml. of heparin solution was injected into the jugular vein, and as much blood as possible was withdrawn from the vein by means of a 2.0 ml. syringe to which was attached a No. 23 needle. In most cases, the liver, kidneys, spleen, gastrointestinal tract and contents, lungs, and femurs were excised, freed of extraneous tissue, and placed in weighing bottles. The remainder of the carcass was placed in a weighed beaker. The tissues were cut into small pieces with scissors and weighed. Determinations of thorium were carried out on two aliquot portions of each tissue. The femurs were dissolved in hot nitric acid and thorium determinations were carried out on aliquot portions of the solution. The carcasses were treated in a similar manner.

Table IV is a summary of the results obtained in an experiment in which the excretion and distribution of thorium were determined for five mice, each of which was given orally 0.25 ml. of a solution of thorium nitrate containing 10 mgm. of thorium per ml. The distribution of thorium was determined seven days after dosing.

Table V is a summary of the results obtained in an experiment in which 0.25 ml. of a thorium nitrate solution containing 100 mgm. of thorium per ml. was administered orally to each of 10 mice. Five of the mice were sacrificed 24 hr. after dosing and five were sacrificed seven days after dosing.

The data obtained in these experiments suggest that all the thorium administered orally to mice as thorium nitrate is excreted by way of the feces with no absorption from the gastrointestinal tract. If this is the case the question arises as to why the administration of even large doses of thorium nitrate to mice results in their death. The following experiment threw some light on this question. Five mice were each given orally 0.25 ml. of a solution of thorium nitrate containing 100 mgm. of thorium per ml. The mice were sacrificed 24 hr. later and the gastrointestinal tracts were excised, opened, and cut in small pieces. This material was placed in a closed cheesecloth bag through which water was allowed to run for two hours. At this time, radioactivity measurements of the wash water showed that no more thorium was

being removed. The thorium content of the gastrointestinal tracts was then determined and was found to be 1.0% of the thorium administered to the mice. It is probable that this thorium had reacted with the proteins of the

TABLE IV

THE EXCRETION AND DISTRIBUTION OF THORIUM IN MICE SEVEN DAYS AFTER THE ADMINISTRATION OF 100 MGM. OF THORIUM (AS THORIUM NITRATE) PER KGM. BODY WEIGHT

Material analyzed	Administered thorium, %
Feces, Day 1	38.0
" " 2	34.0
" " 3	22.0
" " 4	1.2
" " 5	0.0
" " 6	0.0
" " 7	0.0
Total feces	95.2
Total urine	< 0.3
Total urine residue	0.39
Gastrointestinal tract	< 0.03
Liver	"
Kidneys	"
Spleen	"
Lungs	"
Femurs	"
Blood	"
Total in whole animal	< 0.2
Total recovery	95.59

mucosa. It is suggested that thorium nitrate owes its oral toxicity to its local action on the mucosa of the gastrointestinal tract. In the previous experiments, any thorium that reacted with the constituents of the mucosa was excreted in a few days. This may have been a result of sloughing off and excretion of the mucosa that was damaged by the thorium.

An experiment was carried out to determine the distribution and excretion of thorium in mice after the subcutaneous injection of thorium nitrate, and to discover whether the application of BAL (2,3-dimercaptopropanol) had any effect on this. In this experiment the hair of the back and flanks of each of a group of 16 mice was removed by clippers. A 0.5 ml. syringe to which was attached a No. 23 needle was filled with a thorium nitrate solution containing 2.0 mgm. of thorium per ml. The needle of the syringe was inserted under the skin of the right flank of a mouse and 0.25 ml. of the solution was injected. A clamp was then applied to the skin over the needle in such a manner as to prevent the solution from flowing back. The needle was withdrawn and a

small amount of "Glyptal" cement was applied over the hole in the skin left by the needle. When the cement was dry the clamp was removed. This technique prevented any of the solution from flowing to the surface of the

TABLE V

THE EXCRETION AND DISTRIBUTION OF THORIUM IN MICE AFTER THE ORAL ADMINISTRATION OF 1000 MGM. OF THORIUM (AS THORIUM NITRATE) PER KGM. BODY WEIGHT

Material analyzed	Administered thorium, %	
	In Group I sacrificed 24 hr. after dosing	In Group II sacrificed 7 days after dosing
Feces, Day 1	34.7	50.5
" " 2	—	36.5
" " 3	—	8.1
" " 4	—	0.13
" " 5	—	0.08
" " 6	—	0.07
" " 7	—	0.05
Total feces	34.7	95.43
Total urine	< 0.01	< 0.03
Total urine residue	0.06	0.80
Gastrointestinal tract and contents	56.3	< 0.03
Liver	< 0.03	< 0.03
Kidneys	"	"
Spleen	"	"
Lungs	"	"
Femurs	"	"
Blood	"	"
In remainder of carcass	< 0.2	< 0.2
Total recovery	91.06	96.23

skin. The treatment with BAL was carried out immediately after the injection of the thorium nitrate. A dose of 0.3 ml. of BAL, which had a sulphydryl content of 92% of theoretical by iodine titration, was applied to the skin of the left side of the back of the mouse over an area of about 6 sq. cm. The BAL was rubbed in for about three minutes with a glass rod. Sixteen mice were treated with thorium nitrate and eight of these were treated with BAL. Four of the mice that were treated with BAL and four of the mice that were not so treated were sacrificed after 24 hr. and the other eight mice were sacrificed after seven days. Thorium determinations were carried out on the excreta and the internal organs of each group of mice. The amount of thorium in the skin of each mouse at the site of injection of the thorium nitrate and the amount of thorium in each carcass was determined separately. The results of this experiment are summarized in Tables VI and VII. Mean values are quoted with the standard error of the mean.

The data of Tables VI and VII show no evidence that thorium was transported to the viscera after the subcutaneous injection of thorium nitrate and very little of the thorium was excreted. Most of the thorium appears to be

TABLE VI

THE DISTRIBUTION OF THORIUM AFTER THE SUBCUTANEOUS INJECTION OF THORIUM NITRATE SOLUTION IN MICE TREATED WITH BAL AND IN UNTREATED MICE

Values are expressed as percentages of the administered thorium (0.5 mgm. per mouse)

Tissue	Time after dosing: 24 hr.		Time after dosing: 7 days	
	Mice treated with BAL	Untreated mice	Mice treated with BAL	Untreated mice
Skin at the site of injection	74 ± 6	77 ± 5	74 ± 6	80 ± 6
Carcass	21 ± 6	20 ± 6	15 ± 6	15 ± 5
Kidney	<0.05	<0.05	<0.05	<0.05
Liver	"	"	"	"
Spleen	"	"	"	"
Lungs	"	"	"	"
Gastrointestinal tract	"	"	"	"
Blood	"	"	"	"
Femurs	"	"	"	"

TABLE VII

THE EXCRETION OF THORIUM AFTER THE SUBCUTANEOUS INJECTION OF THORIUM NITRATE SOLUTION IN MICE TREATED WITH BAL AND IN UNTREATED MICE

Values are expressed as percentages of the administered thorium (0.5 mgm. per mouse)

Day after dosing	Mice treated with BAL			Untreated mice		
	Urine	Urine residue	Feces	Urine	Urine residue	Feces
1	<0.1	0.03	0.18	<0.1	0.02	<0.1
2	<0.1	0.07	0.53	<0.1	<0.01	<0.1
3	<0.1	0.01	0.22	<0.1	0.01	<0.1
4	<0.1	0.01	<0.1	<0.1	<0.01	<0.1

fixed at the site of injection. The thorium found in the carcass was probably fixed to the subcutaneous tissue beneath the site of injection. The application of BAL did not significantly alter the distribution of thorium in the mice, but did appear to cause a small increase in the excretion of thorium in the feces.

An experiment was carried out to determine the distribution and excretion of thorium in mice after a single exposure to an aerosol of thorium nitrate solution. Twenty female mice were placed in a wire cage with an individual compartment for each mouse and the cage was placed in a closed cubical

chamber of 125 liter capacity. Three ml. of a solution of thorium nitrate containing 10 mgm. of thorium per ml. and with a UX_1 activity of about 9.4 μc. per ml. was placed in each of three small glass atomizers. These atomizers were of the type with a glass baffle, and were obtained from the Vaponefrin Co. The thorium nitrate solution in each atomizer was sprayed into the chamber through a port near the top using an air pressure of 18 lb. per sq. in. An attempt was made to determine the approximate size of the droplets of solution by placing microscope slides in the chamber for short intervals and measuring the size of the droplets on the slides by means of a microscope with a calibrated scale in the eyepiece. It was concluded that the diameter of almost all the droplets was less than 3 μ, the majority being less than 1 μ.

All the thorium nitrate solution was sprayed into the chamber 35 min. after the start of the spraying and the mice were removed from the chamber 180 min. after the start of the spraying. Table VIII shows the concentration

TABLE VIII

THE CONCENTRATION OF THORIUM IN THE AIR OF THE CHAMBER IN WHICH MICE WERE EXPOSED TO AN AEROSOL OF THORIUM NITRATE SOLUTION

Time after the start of the spraying, min.	Concentration of thorium, mgm. of thorium/cu. m. of air
15	82
35	107
50	64
65	42
80	30
95	19
110	15
180	3

of thorium in the air of the chamber at various intervals after the start of the spraying. Each of these determinations was carried out by withdrawing a 2 liter sample of air from a port near the bottom of the chamber. The sample of air was passed at the rate of 0.5 liter per minute through a gas washing bottle containing 25 ml. of water and the thorium content of the water was determined. Previous experiments had shown that all the thorium in the sample of air was absorbed by the water in the absorber under these conditions. Since the port through which the air samples were withdrawn was at the same level as the mice, it is probable that the values quoted in Table VIII are accurate estimations of the concentration of thorium in the air that the mice were breathing.

Groups of four mice each were sacrificed at the following times after the exposure: 5 min., 24 hr., 7 days, 21 days, and 35 days. Each mouse in the group was treated in the following manner at the time of sacrifice. The

blood was withdrawn and the skin was removed as completely as possible. The tail and paws to which some skin adhered were added to the skin. The head without any skin, the lungs, gastrointestinal tract, liver, kidneys, spleen, and femurs were excised. Thorium determinations were carried out on the organs and the remainder of the carcasses of the four mice in the group. Four of the mice were placed in a metabolism cage immediately after the exposure and urine and feces were collected for seven days after the exposure. The results of these experiments are summarized in Tables IX and X.

TABLE IX

THE DISTRIBUTION OF THORIUM IN MICE AFTER EXPOSURE OF THE MICE TO AN AEROSOL OF THORIUM NITRATE SOLUTION

Results expressed as μgm. of thorium in the tissues of one mouse

Tissue	Time after the start of exposure				
	3 hr.	24 hr.	7 days	21 days	35 days
Skin, tail, and paws	149	24.6	3.5	1.6	<0.7
Head without skin	14.8	1.1	<0.5	<0.5	<0.5
Lungs	3.10	2.60	2.25	1.83	1.38
Gastrointestinal tract and contents	85.6	7.4	<0.5	<0.5	<0.5
Liver	<0.2	<0.2	<0.2	<0.2	<0.2
Kidneys	"	"	"	"	"
Spleen	"	"	"	"	"
Femurs	"	"	"	"	"
Blood	"	"	"	"	"
Remainder of carcass	4.7	<1.0	<1.0	<1.0	<1.0
Total	257	36	6	3	1

TABLE X

THE EXCRETION OF THORIUM AFTER THE EXPOSURE OF MICE TO AN AEROSOL OF THORIUM NITRATE SOLUTION

Results expressed as μgm. of thorium excreted by one mouse

Day after the exposure	Urine, μgm. Th	Feces, μgm. Th
1	<0.2	152
2	<0.1	51
3	<0.2	16
4	<0.1	13
5	<0.1	1
6	<0.1	3
7	<0.1	2
Total	<0.9	238

The data in Tables IX and X show no evidence that thorium reaches the liver, kidney, spleen, bone, or blood after exposure of mice to an aerosol of thorium nitrate. It is probable that the small amount of thorium found in the carcasses of the mice five minutes after exposure owed its presence there to contamination from the fur or gastrointestinal tract and that no thorium was actually present in the skeletal muscle or bones of the intact mice. A comparatively large amount of thorium was deposited on the fur of the mice and it is probable that the mice transferred this thorium to the gastrointestinal tract by licking. This thorium was then excreted by way of the feces and very little or no absorption of the thorium took place. The thorium found in the head was probably in the upper parts of the respiratory and gastrointestinal tracts. Almost all of the thorium in and on the mouse was eliminated by way of the feces within a week after the exposure. A small amount of thorium was found in the lungs and this decreased slowly over a period of 35 days after the exposure. It is probable that this thorium reacted with the constituents of the lung tissue.

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"DROP PLATE" METHOD OF COUNTING VIABLE BACTERIA[1]

By R. W. Reed[2] and G. B. Reed[3]

Abstract

This paper is a critical review of the "drop plate" method of determining the number of viable bacteria in fluids, together with a description of an experimental comparison of the "drop plate" with the more usual "pour plate" procedure. It is shown that counts on pure cultures of bacteria made by the "drop plate" method are some 7% higher than those made on the same cultures by the "pour plate" method. It is also shown that the standard error of a series of counts made by the "drop plate" procedure is slightly less than for those made by the "pour plate" method.

A successful "drop plate" method of determining the number of viable bacteria in fluid suspension has been in use for a number of years in several English research laboratories. Considerable experience with the method suggests the desirability of reviewing the procedure both as a research tool and for routine assays of such material as milk.

Donald (2) introduced a method for the precise measurement of fluid volume by means of drops. This depends upon the fact that the size of a drop delivered from a pipette is governed, other factors being constant, by the *external* diameter of the dropping tube. Fildes and Smart (3) expanded the procedure and developed methods of preparing and calibrating the pipette. Several authors have described in brief form the adaptation of dropping pipettes to the technique of plate counts of bacteria, particularly Wilson (9), Aitken, Barling, and Miles (1), Kenny, Johnston, von Haebler, and Miles (5), von Haebler and Miles (4), Miles, Misra, and Irwin (7), and Snyder (8). The last two papers include a statistical analysis of the accuracy of the method.

The plating procedure consists simply in adding, with a calibrated dropping pipette, drops of properly diluted suspensions of bacteria to the surface of nutrient agar or other appropriate medium in Petri plates. The surface of the medium must be dried to the stage where a drop of bacterial suspension will spread over an area of 1.5 to 3 cm. in diameter and permit absorption of the fluid of the drop in 15 to 20 min. On incubation the area covered by a drop of suitably diluted suspension will develop well spaced surface colonies that permit rapid and accurate counts.

Methods

Dropping Pipettes

These are made, as described by Donald, by drawing a length of 7 mm. glass tubing to form two pipettes with long, very gradual tapers. They may

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be sized with a wire gauge; however, we find more convenient a square of steel $\frac{1}{8}$ in. thick with one hole 0.047 in. in diameter. The tapered portion of the pipette is pushed through the gauge hole until it fits snugly, scratched with a sharp file or glass knife at the margin of the gauge, removed, and broken. The external diameter of the pipette should then be very close to that of the gauge, provided the glass is drawn with a very gradual taper and is circular in cross section. The tip must be uniform and at right angles to the axis of the pipette. With practice these may be made rapidly and accurately.

The pipettes are manipulated with a rubber teat and must be held in a perpendicular position when drops are delivered. They may be calibrated by dropping a counted number of drops into a tared weighing bottle. In this procedure, as indicated by Fildes and Smart (3), it is essential to deliver not more than 30 drops a minute. Pipettes made with the 0.047 in. gauge deliver 40 drops of water per ml.

A set of pipettes may be used repeatedly provided they are scrupulously cleaned. Our practice both with the dropping pipettes and with graduated diluting pipettes is to discard used pipettes into Roccal solution, wash them in running water in an automatic washer, allow them to stand for one to two hours in chromate cleaning mixture, and wash them overnight in running water. They are then dried, loosely plugged with cotton, and sterilized in the ordinary manner.

Plating Medium

Several types of media were used in the experiments reported in this paper. In order to prepare different lots as uniform as possible each type was made from accurately weighed amounts of Difco dehydrated medium bearing the same 'batch' numbers.

Drying Plates

The time and temperature of drying the plates must vary with the relative humidity of the atmosphere. In the greater part of this work, with a relative humidity of 60 to 75%, optimum surfaces were obtained by drying recently poured and cooled plates, 20 ml. agar per plate, for one hour at 37° C. In a few cases where the relative humidity ranged from 75 to 100%, satisfactory results were obtained by drying for one hour at 75° C. In all work reported in this paper, plates were dried, tilted upside down on their lids in the drying chamber.

This drying time was arrived at from the results of the experiment summarized in Table I, on counts on a fluid culture of *Shigella alkalescens*. Plates were poured, 15 ml. and 25 ml. per plate and dried, as just described, at 37° for 30, 60, and 90 min. It is apparent from the table that drying within this range has no significant influence on the counts.

TABLE I

EFFECT OF AMOUNT OF AGAR AND DRYING TIME OF PLATES ON DROP COUNT OF *Shigella alkalescens*

	Counts, × 10^8				
15 ml. of agar per plate			25 ml. of agar per plate		
Drying time at 37° C., min.					
30	60	90	30	60	90
9.12	9.06	9.50	9.54	8.66	8.86
8.36	8.40	9.74	8.96	8.50	9.10
8.70	8.50	8.76	9.06	9.16	8.80
Mean 8.73	8.65	9.33	9.19	8.77	8.92
Standard error 0.18	0.17	0.23	0.15	0.16	0.07

Dilutions

The number of colonies developing per drop of fluid added to the plates that can be most satisfactorily counted is 20 to 40. The aim, therefore, in making dilutions is to cover the range of 20 to 40 organisms per drop, i.e. per 1/40th ml. Ordinarily serial 1–10 dilutions were made, or for the most accurate work, especially where the approximate range of concentration of bacteria was known, serial 1–2 dilutions were made. The number of these dilutions used for plating depends on how accurate an estimate can be made of the concentration of bacteria present.

Dilutions were made by adding 1 ml. to a 9 ml. blank in a 6 × $\frac{3}{4}$ in. test tube and mixing, with the pipette, used for the next transfer, by alternately filling and blowing out 10 times. Two fluids were tried for dilution blanks, distilled water and buffered gelatin–saline*. In a number of parallel counts from a single culture of *Shigella alkalescens* using the two dilution fluids the following total counts were obtained:

Diluting fluid	Mean count per ml. of culture
Distilled water	4.10 ± 0.18 × 10^8
Gelatin–saline	4.47 ± 0.20 × 10^8

In all subsequent counts recorded in this paper gelatin–saline was used for the dilutions.

* *Sodium chloride* *0.8 gm.*
Sodium dipotassium phosphate *0.2 gm.*
Gelatin *0.1 gm.*
Distilled water *100 ml.*

Plating

After the dilutions are made and plates of appropriate medium dried, drops of the selected dilutions are added to the plates with calibrated dropping pipettes. Usually six drops of one dilution are added to a plate of agar. In delivering measured drops the most satisfactory procedure is to hold the pipette in a perpendicular position with the tip about 5 mm. above the surface of the agar. It is essential that the pipette be held firmly in a fixed position so that the drops form their maximum size and fall freely from the tip of the pipette. The drops should be evenly spaced on the agar so that they neither coalesce nor extend to the margin of the plate. Within one to two minutes after the drops are delivered the plates should be rotated while firmly held to the bench surface, so as to spread the drop over an area of 1.5 to 3 cm. in diameter. The plates are allowed to stand on the bench in an upright position for 15 to 20 min. to permit absorption of the fluid by the agar. They are then inverted and incubated 16 to 20 hr. or longer in the case of slow growing species.

Counting

Counts are conveniently made with the aid of a colony counter in the ordinary manner. The total number of bacteria per ml. or per gm. of the original material $= \frac{A}{B} \times C \times D$

Where A = number of colonies per plate
B = number of drops per plate (usually 6)
C = number of drops per ml. (usually 40)
D = the dilution factor

Accuracy of Method

A number of experiments were carried out to test the accuracy of the drop method and to compare it with the usual pour plate method of counting.

Six operators having varying degrees of experience with the drop count procedure each prepared a series of dilutions to 1–400,000 in saline–gelatin blanks from the same 24-hr. broth culture of *Salmonella typhimurium*, then prepared six nutrient agar plates, six drops per plate, using three dropping pipettes. Results are shown in Table II. The mean values obtained by the six operators vary from 7.49 to 8.35 $\times 10^8$ and the standard errors of each mean vary from 0.11 to 0.24 $\times 10^8$. It is therefore evident that the difference between the mean values is the order of five times the standard error of each operator's counts.

Comparison of "Drop" and "Pour" Counts

A series of counts were made by five operators on cultures of *Shigella alkalescens*, *Shigella dysenterae*, and *Salmonella typhimurium*. In each instance the five operators worked from the same 24-hr. broth culture. Each operator

TABLE II

DROP PLATING OF *S. typhimurium* ON BACTO-NUTRIENT AGAR BY SIX OPERATORS OF VARYING DEGREES OF EXPERIENCE. DILUTION 1 : 400,000

Operator	Counts, × 10^8					
	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
	8.00	7.80	8.08	7.41	7.17	8.14
	7.48	7.57	7.68	8.04	7.64	9.39
	9.31	6.93	7.54	8.18	7.92	8.24
	8.14	8.12	8.24	8.04	7.20	7.80
	8.77	8.64	7.80	7.34	7.96	7.73
	8.28	7.48	—	7.84	7.06	8.82
Mean	8.33	7.76	7.97	7.81	7.49	8.35
Standard error	0.24	0.22	0.11	0.13	0.13	0.24

NOTE: *Mean count, all plates = 7.95 × 10^8.*
Standard error = 0.051 × 10^8.

made one set of dilutions and prepared "drop" and "pour" plates from this one set of dilutions. The same lot of medium was used for the two types of plates. Differences between the counts by the two methods must therefore result from the actual plating and growth on the plates rather than from diluting procedures. The results are summarized in Tables III to V.

TABLE III

COMPARISON OF "DROP" AND "POUR" PLATING OF *Shigella alkalescens* ON BACTO-NUTRIENT AGAR BY FIVE OPERATORS. DROP PLATES MADE FROM 1 : 500,000 DILUTION, POUR PLATES FROM 1 : 3,000,000

Operator	Counts, × 10^8									
	No. 1		No. 2		No. 3		No. 4		No. 5	
	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"
	5.34	5.19	5.62	6.03	5.60	5.22	6.00	4.05	5.14	5.76
	5.66	5.01	5.20	5.31	4.86	6.21	6.46	4.56	5.60	4.98
	4.74	4.50	5.36	5.94	5.40	5.52	5.56	4.77	5.14	4.77
	5.40	5.91	6.30	5.52	5.16	5.37	4.70	4.29	4.90	5.34
	5.36	5.04	5.96		5.54	5.58	4.42	4.02	5.60	5.55
	4.56	5.31	4.62		5.40	5.19	5.30		4.80	4.92
Mean	5.18	5.16	5.51	5.56	5.33	5.52	5.41	4.42	5.20	5.22
Standard error	0.16	0.17	0.22	0.16	0.09	0.14	0.29	0.12	0.13	0.15

	Mean	Standard error
30 "Drop" plates	5.33 × 10^8	0.022 × 10^8
27 "Pour" plates	5.18 × 10^8	0.078 × 10^8

TABLE IV

COMPARISON OF "DROP" AND "POUR" PLATING OF *Shigella dysenteriae* ON BACTO-NUTRIENT AGAR BY FIVE OPERATORS. DROP PLATES MADE FROM 1 : 500,000 DILUTION, POUR PLATES FROM 1 : 3,000,000

Operator	Counts, $\times 10^8$									
	No. 1		No. 2		No. 3		No. 4		No. 5	
	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"
	4.96	5.13	4.80	4.56	5.96	5.31	4.60	5.46	5.36	4.74
	5.00	5.40	5.96	4.35	5.90	4.92	5.06	5.10	5.40	5.67
	5.54	5.43	6.10	4.50	6.16	5.52	4.70	4.17	4.42	4.89
	5.34	4.95	5.26	4.77	5.66	5.43	4.22	4.29	5.20	4.71
	5.94	4.89	6.10	4.74	4.70	5.16	5.56	4.44	5.42	6.24
	5.54	5.01	5.10	5.13	5.34	4.89	5.16	4.56	5.06	5.13
Mean	5.39	5.15	5.57	4.68	5.62	5.21	4.88	4.67	5.14	5.23
Standard error	0.14	0.09	0.21	0.11	0.19	0.10	0.17	0.19	0.14	0.22

	Mean	Standard error
30 "Drop" plates	5.32×10^8	0.051×10^8
30 "Pour" plates	4.95×10^8	0.048×10^8

TABLE V

COMPARISON OF "DROP" AND "POUR" PLATING OF *Salmonella typhimurium* ON BACTO-NUTRIENT AGAR BY SIX OPERATORS. DROP PLATES MADE FROM 1 : 500,000 DILUTION AND POUR PLATES FROM 1 : 3,000,000

Operator	Counts, $\times 10^8$											
	No. 1		No. 2		No. 3		No. 4		No. 5		No. 6	
	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"	"Drop"	"Pour"
	7.14	6.72	6.74	6.00	6.54	5.52	6.40	6.66	7.82	7.20	7.62	7.68
	7.36	7.23	7.22	6.84	6.54	4.83	6.54	6.33	6.94	6.60	7.46	7.05
		6.90	7.70	6.30	7.56	4.56	7.36	6.06	7.40	6.87	7.40	6.41
		7.59	7.02	6.27	7.26	6.12		5.52	7.56	7.71		6.84
		6.96	7.10	5.73		5.85		7.23	6.76	7.23		5.67
		7.53	7.34	5.67		5.46		6.18	7.26	6.84		7.11
			7.66									
Mean	7.25	7.12	7.25	6.14	7.47	5.39	6.77	6.33	7.29	7.08	7.49	6.79
Standard error	0.08	0.13	0.12	0.16	0.34	0.17	0.24	0.21	0.15	0.15	0.06	0.26

	Mean	Standard error
23 "Drop" plates	7.25×10^8	0.05×10^8
36 "Pour" plates	6.48×10^8	0.10×10^8

The five operators, Table III, obtained "drop" counts on one culture of *Shigella alkalescens* with mean values of 5.18×10^8 to 5.51×10^8 and with standard errors of 0.14 to 0.21×10^8 per ml. of culture, and "pour" counts with mean values of 4.42×10^8 to 5.56×10^8 with standard errors of 0.09 to 0.22×10^8. Similar relative values were obtained by the two methods of counting cultures of *Shigella dysenterae*, Table IV, and *Salmonella typhimurium*, Table V. In the summaries at the bottom of Tables III to V it will be noted that in each instance the mean of the "drop" count is 3 to 10% higher than the mean of the "pour" count. It will also be noted from these summaries that in each instance the standard error of the mean of the "drop" counts is slightly less than the standard error of the mean of the "pour" counts.

Counts made on two lots of *Bacillus subtilis* spores stored in semi-dry fine ground peat (Lochhead and Thexton (6)) for 15 and 24 months and for 12 and 19 months were made on Difco heart infusion agar and on the regular laboratory heart infusion agar. As in former determinations the counts shown in Table VII indicate mean values for the "drop" plate counts slightly higher than the means of the "pour" plate counts but counts on another lot of similar material, shown in Table VI, indicate the reverse, i.e., mean values of the

TABLE VI

COMPARISON OF "DROP" AND" POUR" PLATING OF *Bacillus subtilis* ON TWO TYPES OF AGAR AFTER 15 AND 24 MONTHS' STORAGE IN PEAT AT 28% MOISTURE

Counts, $\times 10^8$

	15 months storage in peat				24 months storage in peat			
	Drop plates		Pour plates		Drop plates		Pour plates	
	1 : 4,000,000 dil.		1 : 20,000,000 dil.		1 : 2,000,000 dil.		1 : 20,000,000 dil.	
	Difco infusion	Lab. infusion	Difco infusion	Lab. infusion	Difco infusion	Lab. infusion	Difco infusion	Lab. infusion
	2.96	3.39	3.28	3.06	2.49	3.11	4.56	3.50
	3.07	2.99	3.28	3.00	3.97	3.24	3.66	4.20
	3.31	3.76	3.16	2.94	3.40	3.43	4.20	3.78
	3.36	3.39		2.24	3.86	2.93	4.16	3.64
	3.07	3.07		3.12	3.13	2.77	3.78	3.74
					3.66	2.99	3.68	3.54
Mean	3.15	3.32	3.24	2.87	3.42	3.08	4.01	3.73
Standard error	0.07	0.12	0.03	0.15	0.20	0.08	0.13	0.09

"drop" plate counts are slightly lower than the mean values of the "pour" plate counts. This is the only case in many comparative counts where the "drop" plate counts have not been higher than the "pour" plate counts. It is apparent from the tables that the standard error of the two methods of counting *B. subtilis* is of the same order.

TABLE VII

COMPARISON OF "DROP" AND "POUR" PLATING OF *Bacillus subtilis* ON TWO TYPES OF AGAR AFTER 12 AND 19 MONTHS' STORAGE IN PEAT AT 25% MOISTURE

	Counts, $\times 10^8$							
	12 months storage in peat				19 months storage in peat			
	Drop plates		Pour plates		Drop plates		Pour plates	
	1 : 600,000 dil.		1 : 5,000,000 dil.		1 : 300,000 dil.		1 : 3,000,000 dil.	
	Difco infusion	Lab. infusion	Difco infusion	Lab. infusion	Difco infusion	Lab. infusion	Difco infusion	Lab. infusion
	12.32	13.60	10.45	12.75	3.86	3.94	2.55	2.49
	13.48	13.60	11.30	11.35	3.06	3.28	4.23	3.09
	11.80	12.04	12.15	10.00	3.90	3.64	3.78	3.03
	11.72	11.56	11.50	10.90	3.46	3.56	3.60	2.97
	12.20	13.24	10.70	11.85	3.94	3.28	3.12	2.76
	11.08	13.28	9.75	9.85	3.90	3.70	3.48	2.76
					3.48	3.46	3.63	3.30
					3.64	3.22		
Mean	12.10	12.55	10.98	11.12	3.66	3.52	3.48	2.91
Standard error	0.30	0.35	0.31	0.41	0.09	0.08	0.18	0.09

Counts on Differential Media

Counts were made on cultures of *Shigella alkalescens*, *Shigella dysenterae*, and *Salmonella typhimurium* on blood agar, MacConkey's, Endo's, and Difco S.S. agar in contrast with Difco heart infusion and laboratory heart infusion agars primarily to determine the significance of counts on differential media.

It is apparent from the results shown in Tables VIII, IX, and X that consistent counts may be made by the "drop" method on opaque media. It is

TABLE VIII

COMPARISON OF "DROP" AND "POUR" PLATING OF *Shigella alkalescens* ON SEVERAL TYPES OF BACTO AGAR. "DROP" PLATES FROM 1:500,000 DILUTION, "POUR" PLATES FROM 1 : 3,000,000

	Counts on drop plates, $\times 10^8$					Counts on pour plates, $\times 10^8$	
	Difco infusion	Lab. infusion	Blood	Mac-Conkey	Endo	Difco infusion	Lab. infusion
	5.10	5.14	5.36	5.76	5.20	4.32	4.77
	5.70	5.60	5.44	4.56	5.60	5.55	5.76
	5.00	5.14	5.50	5.74	4.84	4.86	4.98
	5.02	4.90	5.66	5.20	4.50	5.31	5.34
	4.42	5.60	5.10	4.94	5.16	4.53	5.55
	5.62	4.80	5.96	5.66	4.60	4.47	4.92
Mean	5.14	5.20	5.50	5.31	4.98	4.84	5.22
Standard error	0.18	0.15	0.11	0.19	0.18	0.18	0.15

TABLE IX

COMPARISON OF "DROP" AND "POUR" PLATING OF *Shigella dysenteriae* ON SEVERAL TYPES OF AGAR. "DROP" PLATES MADE FROM 1 : 500,000 DILUTION, "POUR" PLATES FROM 1 : 3,000,000

	Counts on drop plates, × 10^8					Counts on pour plates, × 10^8	
	Difco infusion	Lab. infusion	Blood	Mac-Conkey	S S	Difco infusion	Lab. infusion
	5.56	5.36	6.50	4.96	2.14	6.54	4.74
	5.94	5.40	6.14	5.10	2.54	5.79	5.67
	5.96	4.44	6.50	5.16	1.46	5.19	4.89
	5.96	5.20	5.90	4.96	1.86	4.83	4.71
	5.76	5.44	6.36	3.90	2.04	5.67	6.24
	6.56	4.56	6.46	5.90	2.14	5.61	5.13
Mean	5.96	5.10	6.31	5.00	2.03	5.61	5.23
Standard error	0.12	0.17	0.09	0.23	0.13	0.22	0.23

TABLE X

COMPARISON OF "DROP" AND "POUR" PLATING OF *Salmonella typhimurium* ON SEVERAL TYPES OF AGAR. "DROP" PLATES MADE FROM 1 : 500,000 DILUTION, "POUR" PLATES FROM 1 : 3,000,000

	Counts on drop plates, × 10^8						Counts on pour plates, ×10^8	
	Difco infusion	Lab. infusion	Blood	Mac-Conkey	Endo	S S	Difco infusion	Lab. infusion
	7.06	7.64	7.14	7.70	7.64	4.14	8.01	7.68
	7.80	7.44	6.30	6.96	7.54	3.84	7.74	7.05
	6.86	7.44	7.84	6.04	7.10	4.70	7.26	6.67
	7.16		7.46	6.96	7.16	3.66	6.84	7.14
	7.10		6.96	7.20	8.00	3.74	7.14	5.67
	7.76		7.66	6.46	6.96	3.86	8.38	7.11
Mean	7.29	7.51	7.23	6.88	7.40	3.99	7.48	6.89
Standard error	0.15	0.05	0.26	0.21	0.15	0.14	0.23	0.25

also apparent that with these three species MacConkey's medium gives only slightly lower counts than the standard media but that Endo's and Difco S.S. media give by contrast counts that are too low to be significant.

Duplicate counts made by "drop" and "pour" methods on heart infusion and nutrient agars again show, Tables VIII, IX, and X, that the "drop" method gives slightly higher mean counts than the "pour" method. The tables also show that the standard error of "drop" counts is appreciably lower than the standard error of "pour" counts.

Counts of Other Groups of Bacteria

In addition to the four species mentioned in this paper successful "drop" counts have been carried out on one to several species belonging to the following groups of bacteria.

Group	*Medium*
Staphylococcus	Infusion agar
Streptococcus	Infusion agar
Pneumococcus	Blood agar
Serratia	Infusion agar
Pseudomonas	Infusion agar
Achromobacter	Infusion agar
Proteus	Infusion agar
Salmonella	Infusion agar
Eberthella	Infusion agar
Shigella	Infusion agar
Lactobacillus	Milk agar
Pasteurella	Tryptose agar
Brucella	Tryptose agar
Bacillus	Infusion agar

The method appears to be applicable to any aerobic form. The method has been tried with *Clostridium*, with and without a reducing agent in the plating medium, with the plates incubated in Fildes type anaerobic jars. However, the counts were always much lower than with "pour" plates made from the same agar and incubated in the same anaerobic jar.

Conclusion

It is apparent from these results that the "drop" method of making counts of bacteria has some advantages over the conventional "pour" plate method: it is less laborious, it is a little more accurate, with most of the species tested the counts are appreciably higher, and it is applicable to opaque as well as transparent media.

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COMPARISON OF "DROP" AND "POUR" PLATE COUNTS OF BACTERIA IN RAW MILK[1]

By J. J. R. Campbell[2] and J. Konowalchuk[3]

Abstract

In the preceding paper it was shown that a "drop plate" method of determining the number of viable bacteria in pure cultures gives slightly higher counts than the usual "pour plate" method. In this paper it is shown that, in parallel counts made by the two methods on a series of samples of raw milk, the "drop plate" counts are some 27% higher than the "pour plate" counts. It is suggested that this discrepancy results from the more efficient breaking up of clumps and chains of bacteria by the dilution procedure used in preparing the "drop plates."

In the preceding paper, Reed and Reed (1), the technique of "drop plate" assays for viable bacteria as applied to pure cultures was reviewed and the results compared with those obtained in the conventional "pour plate" method. It was demonstrated that the "drop plate" method is slightly more accurate than the "pour plate" method; that the labor involved in preparing the plates is less; and that colony counts are made with greater certainty in the "drop plate" than in the "pour plate" procedure. Since all these comparisons were made with pure cultures as the plating material it seemed desirable to compare the two methods on some natural product that exhibits a mixed flora. Raw milk was chosen and the following experiments were carried out.

Individual samples of raw milk from 28 different producers were collected as delivered at a local dairy. Duplicate "drop plate" counts were made on each sample by an operator with long experience with the method. In this procedure dilutions were made, as described by Reed and Reed (1) by adding 1 ml. to 9 ml. blanks and mixing by alternately filling and blowing out the pipette 10 times, the pipette used for the next transfer. Conventional "pour plate" counts were made in duplicate on the same samples of milk by an operator with long experience in making routine counts by this method. For the "pour plates" dilutions were made by adding 1 ml. to 99 ml. blanks in square dilution bottles and mixing by three minutes hand shaking. Dilution blanks and agar for both methods were from the same batches of material. Agar prepared according to Standard Methods of milk analysis was used for both sets of plates and incubation of plates was in accordance with Standard Methods. Not more than two to three minutes elapsed between the time a sample of milk was plated by one operator and the time it was plated by the other. The time required to prepare dilutions and the plates was approximately the same for the two methods. Colony counts were made somewhat more rapidly on the "drop plates."

[1] *Manuscript received July 28, 1948.*
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Results summarized in the table indicate that the mean count for all samples plated by the "drop plate" method was 27% higher than that for the same samples plated by standard methods. The mean deviation per sample with duplicate counts is greater with the "drop plate" than with the "pour plate" method. This confirms and extends the conclusions of the previous paper that the "drop plate" method gives higher counts than the conventional method. It is also shown that with a mixed flora, such as occurs in raw milk, one can expect even greater differences in count between the two methods than with pure cultures of the organisms used by Reed and Reed (1).

Since a method that favors only bacteria that thrive in air gives a higher count than the standard plating procedure, even when the sample being assayed contains a predominance of lactic acid bacteria, which are inhibited by air, then there must be more serious limitations to the standard plate count than is now recognized. There are several possible explanations. One is that agar at 43° C. to 45° C. lowers the count more than is believed. Another possible explanation is that many aerobic types are not given an opportunity to grow on a pour plate. However, this should not be true with these samples of milk where the overwhelming majority of the organisms appear to be either lactic acid bacteria or members of the colon aerogenes group. The most reasonable explanation would seem to be that the pipetting technique used in mixing the dilutions in the "drop method" is much more efficient in breaking up chains and clumps of cells than is the usual shaking technique.

TABLE I

COMPARISON OF "DROP" AND "POUR" PLATING OF RAW MILK SAMPLES

Milk sample No.	"Drop plates"			"Pour plates"		
	Count/ml., × 1000	Mean, × 1000	Mean deviation, × 1000	Count/ml., × 1000	Mean, × 1000	Mean deviation, × 1000
1	4800 2800	3800	±1000	2300 2500	2400	100
2	4400 11,000	7700	±3300	2500 2450	2475	25
3	520 600	560	± 40	500 500	500	0
4	320 280	300	± 20	125 140	132.5	7.5
5	68 92	80	12	85 75	80	5
6	20 32	26	6	14.5 15.5	15	0.5
7	28 24	26	2	17.5 18	17.75	0.25
8	4 4	4	0	5.5 6.2	5.85	0.35

Note:	*Drop plates*	*Pour plates*
Mean count per sample	*1,482,280*	*1,077,600*
Mean deviation per sample	*264,140*	*119,000*

TABLE I—*Concluded*

COMPARISON OF "DROP" AND "POUR" PLATING OF RAW MILK SAMPLES—*Concluded*

Milk sample No.	"Drop plates"			"Pour plates"		
	Count/ml., × 1000	Mean, × 1000	Mean deviation, × 1000	Count/ml., × 1000	Mean, × 1000	Mean deviation, × 1000
9	640 440	540	100	1000 1050	1025	25
10	3200 3600	3400	200	4500 5000	4750	250
11	360 320	340	20	25 25	25	0
12	5600 5600	5600	0	3000 3500	3250	250
13	160 160	160	0	40 70	55	15
14	20 16	18	2	7.5 8.0	7.75	0.25
15	16 24	20	4	4.5 5.0	4.75	0.25
16	2800 3600	3200	400	4000 2900	3450	550
17	200 60	130	70	75 100	87.5	12.5
18	160 160	160	0	60 55	57.5	2.5
19	400 280	340	60	420 380	400	20
20	40 40	40	0	33 25	29	4
21	7600 6800	7200	400	1500 1450	1475	25
22	2000 440	1220	780	450 2000	1225	775
23	80 40	60	20	35 35	35	0
24	40 80	60	20	17 33	25	8
25	320 280	300	20	350 240	295	55
26	4800 3600	4200	600	5300 4000	4650	650
27	1300 1900	1600	300	2500 3000	2750	250
28	440 400	420	20	650 1250	950	300

Note:	*Drop plates*	*Pour plates*
Mean count per sample	*1,482,280*	*1,077,600*
Mean deviation per sample	*264,140*	*119,000*

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SURFACE GROWTH OF BACTERIA ON CELLOPHANE[1]

By G. B. Reed[2] and D. G. McKercher[3]

Abstract

A method is described for the cultivation of bacteria on the surface of sheets of cellophane spread over layers of absorbent material as cotton saturated with any desired fluid medium. The method has proved to be useful in preparing suspensions of bacteria reasonably free from contamination by the culture medium.

In the many undertakings that require the surface growth of bacteria in mass, as the preparation of certain antigens, agar is at times a disturbing factor. During the recent period of agar scarcity a procedure was developed that has more recently been used in order to avoid agar. The procedure consists in spreading a sheet of cellophane over a layer of absorbent material saturated with the desired fluid culture medium and cultivating the organisms on the surface of the cellophane.

The method is of most value where larger surfaces than provided by ordinary Petri dishes are required. Enamel baking dishes, 1 to 2 in. deep, with nearly perpendicular sides, with a slightly larger dish used as a cover, as a Petri dish, are satisfactory. For still larger areas ordinary plastic cafeteria trays* have proved convenient. Where only a small number are used each tray may be conveniently enclosed in a heavy paper envelope with an opening at one end. For larger numbers of trays it is convenient to equip a large rectangular autoclave with racks that will permit sliding one tray above another, without covers, in the manner of a chest of drawers. When the autoclave door is opened the trays may be withdrawn individually or pulled out part way, like drawers, for inoculation. Such an autoclave must be further equipped with a thermoregulator that will permit operation as an autoclave at 120° C. and later as a steam heated incubator.**

Various grades of cellophane, with the exception of "moisture proof" are satisfactory. "Plain cellophane,"*** No. 300, which is listed as 0.00088 in. thick or No. 450, 0.0012 in. thick are satisfactory. These may be obtained in sheets cut to size or in rolls cut to any desired width.

Various absorbent materials have been used to hold fluid media and support the layers of cellophane. Filter pulp, as supplied by Reeve Angel in sheets

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* *New trays should be soaked in water overnight and well steamed before use.*

** *Such thermoregulators and steam control valves may be obtained from the Bristol Company of Canada, Limited, Toronto.*

*** *Canadian Industries Ltd., Cellophane Division, Toronto.*

about 1 cm. thick, may be cut to fit the trays, enough fluid medium is added to completely saturate the pulp, the cellophane sheet added and firmly rolled down with a photographic print roller. Absorbent cotton, about half the thickness of the ordinary roll, is equally good when similarly treated. Bran, meal, or peat have also been used with good results. Enough fluid medium is added to the material to make a thick paste that is spread roughly in the tray, a sheet of cellophane is added and rolled smooth. It is not necessary to use clarified fluid medium as it filters through the cellophane.

In one large series of cultures the fluid medium consisted of 7% crude corn steep liquor in water without clarification. This was mixed with the following proportions of crushed peat, as used by Lochhead and Thexton (1), for the storage of bacteria, and asbestos fiber as used for insulation.

For 25 trays (15 × 20 in.)

Crude corn steep liquor	1200 ml.
Tap water	16,000 ml.
Crushed peat	2 kgm.
Asbestos fiber	4 kgm.

The acid corn steep and acid peat are neutralized by the alkaline asbestos to give a well buffered medium, after autoclaving, of pH 7.2 to 7.4.

The only difficulty in handling this material is to prevent curling of the cellophane in the autoclave. This can be avoided by preventing the formation of a vacuum as the autoclave cools. A water manometer should be attached to the autoclave. In most autoclaves this can be done very simply by introducing a T in the pipe leading to the pressure gauge and providing the side arm with a valve and a small glass manometer. In operation, as the autoclave cools and the pressure gauge approximates zero, the valve to the manometer may be cracked momentarily at short intervals until the manometer just registers zero. The air port on the autoclave is then opened; this prevents vacuum formation on further cooling. Incidently the procedure is of value in the preparation of coagulated serum or egg slants.

The cellophane surface may be inoculated in a variety of ways. A few drops of inoculum added with a pipette spreads readily with an angle glass rod or the angle rod may be wrapped with gauze and kept moist by adding inoculum drop by drop.

The period of growth and the method of harvesting will vary with the type of organism. Cells of more butyrous growths or the almost woolly growths of some species of *Bacillus*, before sporulation is complete, can be readily harvested by scraping the surface of the cellophane with a square of glass, as a lantern slide cover, and pushing the mass of cells from the edge of the glass into a beaker with a glass spatula. Cell masses in more watery growths, as most *Salmonella* species or spores of *Bacillus* after the vegetative rods have

autolyzed, may be more readily collected with a vacuum device. Such a device may be made of about $\frac{1}{4}$ in. stainless steel tubing in the form of a T with the cross arm 3 to 4 in. long carrying a very narrow slit, 0.2 to 0.5 mm. The long arm of the T, which serves as a handle, is connected by rubber tubing to a receiving vacuum bottle. It is readily possible by this method to collect vegetative cells or spores in the form of a thin paste with the order of 10^{10} to 10^{12} cells per ml.

Reference

1. LOCHHEAD, A. G. and THEXTON, R. H. Can. J. Research, C, 25 : 1. 1947.

METHODS FOR THE DETERMINATION OF THE DISTRIBUTION OF RADIOACTIVE PHOSPHORUS AMONG THE PHOSPHORUS-CONTAINING CONSTITUENTS OF TISSUES[1]

By J. A. McCarter[2] and Ethel L. Steljes[3]

Abstract

The phosphorus-containing constituents of various tissues of the rat were separated into five fractions. These were: inorganic and organic phosphorus soluble in aqueous 10% trichloroacetic acid; phosphorus soluble in 3 : 1 alcohol–ether; phosphorus derived from ribonucleic acid; and phosphorus derived from thymonucleic acid. In order to test the methods, inorganic phosphate, disodium phenyl phosphate, and purified ribonucleic and thymonucleic acids were added to a homogenate of rat liver in 10% trichloroacetic acid. It was possible by separation and analysis of the fractions to obtain a quantitative recovery of the added phosphorus in the appropriate fractions. The extent to which radioactive inorganic phosphate entered the other fractions was measured following its addition to a homogenate of rat liver in 10% trichloroacetic acid. When the fractionation was performed at room temperature it was found that all of the fractions except the organic acid-soluble phosphorus fraction contained negligible amounts of the added radioactive phosphorus. The amount of radioactive phosphorus taken up by this fraction could be reduced to a negligible quantity by working quickly and with cold solutions.

Data are given showing the radioactive and nonradioactive phosphorus contents of the various fractions of liver, spleen, kidney, muscle, thymus gland, lymph node, prostate gland, and testis of the rat 24 hr. after the intraperitoneal injection of a solution of radioactive inorganic phosphate. By analysis of the urine, feces, and remainder of the rat after removal of the above tissues, a quantitative account was made of the injected radioactive phosphate.

Introduction

Other investigators (9, 11) have reported methods for the determination of the distribution of the phosphorus of tissues among the phosphorus-containing constituents but these methods were not well suited to the purposes of the present work. The methods used in this laboratory permit the separation of inorganic and organic phosphorus soluble in 10% trichloroacetic acid, phosphorus soluble in 3 : 1 alcohol–ether, phosphorus derived from ribonucleic acid, and phosphorus derived from thymonucleic acid. The methods used for these separations were based on procedures described by Le Page and Umbreit (6), Delory (2), Bloor (1), and Schmidt and Thannhauser (8). When suitably modified it was possible to combine these procedures to permit the determination of the nonradioactive phosphorus (hereafter referred to as P^{31}) and radioactive phosphorus (P^{32}) contents of each of the above groups of substances present in a small sample of tissue.

The procedure involved the grinding of the tissue in aqueous 10% trichloroacetic acid, boiling the residue with a mixture of 3 : 1 alcohol–ether under

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reflux, treating the extracted residue with 1 *N* sodium hydroxide at 35° C. and acidifying the resulting solution in order to separate ribonucleotide phosphorus and thymonucleotide phosphorus.

The procedures were tested by adding known amounts of phosphorus as inorganic phosphate, disodium phenyl phosphate, purified sheep liver ribonucleic acid, and calf thymus nucleic acid to a homogenate of rat liver in 10% trichloroacetic acid. This was done in order to enrich the corresponding phosphorus fractions of the tissue. Fractionation of the homogenate before and after the addition of the above substances and analysis of the fractions resulted in a recovery of over 90% of the phosphorus of each of the added substances in the corresponding fraction.

Before the procedures could be applied to the determination of the distribution of P^{32} among the various fractions it was necessary to determine to what extent exchange of P^{32} took place between them while they were being separated. In order to do this P^{32} as inorganic phosphate was added to a homogenate of normal rat liver in 10% trichloroacetic acid at room temperature and the various fractions were separated and analyzed. It was found that the organic acid-soluble phosphorus fraction contained approximately 10%, the alcohol–ether extract contained 0.004%, the ribonucleotide phosphorus fraction contained 0.004%, and the thymonucleotide phosphorus fraction contained 0.001% of the added P^{32}. When the experiment was repeated with care to work quickly and to keep the trichloroacetic acid solutions cold, the organic acid-soluble phosphorus was found to contain 3.4% of the added P^{32} in one experiment and 2.8% in another. Because it was found necessary to employ cold conditions during part of the fractionation procedure animals were killed by freezing with liquid nitrogen. The tissues were removed and were kept frozen until they were ground in cold trichloroacetic acid.

Tissues of the rat that have been analyzed by the procedures described in this paper are muscle, liver, spleen, kidney, testis, prostate gland, thymus gland, and lymph node. In order to illustrate the application of the methods, the results of a typical experiment on the uptake of P^{32} by the various fractions of the above tissues of the rat are given in detail in this paper.

Experimental

The determination of nonradioactive and radioactive phosphorus

The present work required the direct determination of the specific activity of a given phosphorus fraction. This was done by preparing a solution of the fraction and determining the P^{31} and P^{32} contents of the solution. Organic material was digested with concentrated nitric and perchloric acids and a portion of the resulting solution was used for the determination of its P^{31} content by King's modification (5) of the method of Fiske and Subbarow (3). Another portion of the same solution was used for the determination of its

P^{32} content by precipitation of ammonium molybdiphosphate and determination of the β-ray activity of the precipitate. Preliminary experiments showed that after the administration of a small dose of P^{32} (10 to 20 μc. (microcuries)) to a rat it would not be necessary to use more than 1 mgm. and rarely necessary to use more than 0.2 mgm. of phosphorus derived from tissue in order to obtain a satisfactory measurement of its radioactivity. In order to ensure that the precipitation of ammonium molybdiphosphate would take place under comparable conditions for every sample it was decided to precipitate P^{32} in association with 1.0 to 1.2 mgm. of phosphorus. It was necessary, therefore, to add nonradioactive phosphorus to every sample that contained less than 1.0 and 1.2 mgm. of P^{31}.

The Determination of Nonradioactive Phosphorus

Inorganic Phosphate

Inorganic phosphorus was determined using the method of King (5) except that the volume of solution at the time of color development was 10.0 ml. instead of 15.0 ml. and the time allowed for color development was 30 min. instead of five minutes. These changes were introduced for purposes of convenience. Neither change was found to influence the results of the analysis to a measurable extent.

Total Phosphorus

The method of King was modified further by digestion of organic material with concentrated nitric acid prior to digestion with perchloric acid. This was done in order to decrease the probability of occurrence of explosions. Alcohol–ether extracts of tissue were evaporated to a small volume in the presence of water before the addition of nitric acid.

The phosphorus content of a large mass of organic material such as feces or the carcass of a rat was determined by placing the material in a large beaker and adding enough concentrated nitric acid to cover it. The beaker was covered with a large watch glass and was warmed on an electric hot plate until frothing had subsided and most of the material had dissolved. The temperature was then raised until the nitric acid boiled and more nitric acid was added as necessary until no visible trace of organic material remained. The process of solution of an entire rat required about 8 to 10 hr. for its completion. Finally the solution was transferred with concentrated nitric acid to a suitable volumetric flask and an aliquot part of this solution was used for the determination of its content of P^{31} and P^{32}.

The Determination of Radioactive Phosphorus

Phosphorus was precipitated as ammonium molybdiphosphate by a method based on that described by Willard and Diehl (12). A suitable volume of the solution to be analyzed was measured into a 40 ml. short-cone heavy-duty Pyrex centrifuge tube. Water was added to make the volume 10 ml. and a sufficient volume of a solution of potassium dihydrogen phosphate was added

to make the amount of P^{31} contained by the sample 1.0 to 1.2 mgm. Enough 72% perchloric acid was added so that the mixture contained 1 ml. of the acid. Two milliliters of a 50% saturated solution of ammonium nitrate was added and the mixture was stirred by agitating the tube. The tube was placed in a water bath at 80° C. for 10 to 15 min. and 5 ml. of 5% molybdic acid reagent* were added slowly with agitation. The tube was removed from the bath and allowed to stand at room temperature for one to two hours with occasional mixing. A small proportion of the precipitate was found to float on the surface of the liquid. It was possible to sink this material by adding a small volume of 95% ethanol to the tube in such a way that a layer of alcohol was formed at the top of the liquid. The precipitate was collected by centrifugation and the supernatant liquid was discarded. The precipitate was washed twice by suspending it in 10-ml. portions of 1 : 50 (v./v.) nitric acid and centrifuging. The supernatant liquids were discarded.

The above procedure was tested by weighing the ammonium molybdiphosphate precipitated from solutions containing 1.0 mgm. of P^{31} as phosphate. The average recovery of P^{31} obtained in six such experiments was 99% (standard deviation = ± 0.8%).

In order to prepare the precipitate for measurement of its radioactivity it was dissolved in a few drops of concentrated ammonium hydroxide and was transferred with water to a small flat-bottomed aluminum dish having a slightly raised edge. The solution was evaporated to dryness under an infrared lamp in such a way that a uniform deposit was obtained over the bottom of the dish.**

In order to determine whether or not a quantitative precipitation of P^{32} could be obtained by the use of the above procedure the following experiment was performed. To a solution of inorganic radioactive phosphate 1 mgm. of P^{31} as phosphate was added and precipitated as ammonium molybdiphosphate. To the supernatant liquid after this precipitation a further 1 mgm. quantity of P^{31} was added. This was also precipitated as ammonium molybdiphosphate in order to carry down any P^{32} left after the first precipitation. The average amount of activity found in the precipitate obtained from the supernatant liquid was equal to 0.32% (standard deviation = ± 0.02%) of that of the first precipitate. This was taken as evidence for the quantitative nature of the precipitation.

* *Five per cent molybdic acid reagent was prepared according to the directions of Willard and Diehl (12).*

** *This technique of preparing the precipitate for measurement of its activity has been replaced by the collection of the precipitate on filter paper in a device similar to the Tracerlab E-8 precipitation apparatus (Tracerlab Inc., Boston, Mass.). The precipitate is washed in the apparatus with 1 : 50 (v./v.) nitric acid and with 95% ethanol. It has been found advantageous to complete the washings with a dilute solution of an alcohol-soluble resin in order to prevent the precipitate from falling off the paper when dry. For this purpose we have used a 4% solution of Gelva V-7 (Shawinigan Chemicals Ltd., Montreal) in 95% ethanol. The paper is sucked dry and is mounted in a suitable holder under the window of the counter tube.*

The measurement of the β-ray activity of the precipitate was made using a Geiger-Mueller counter tube of the end-window type and a "scale of 128".* In order to permit the calculation of the activity of test samples relative to a standard and to avoid the necessity of making corrections for decay of P^{32}, the activities of the test samples were compared with those of several identically prepared and mounted samples obtained from aliquot parts of serial dilutions of the parent specimen of the radioactive phosphate solution. Corrections for differences in back scatter and self-absorption in samples containing 1.0 to 1.2 mgm. of P^{31} were not necessary. In order to calibrate the P^{32} samples in absolute units (μc.) the activities of the standards were compared with that of an identically mounted, weighed quantity of pure U_3O_8 covered with a sheet of aluminum whose thickness was 27 mgm. per sq. cm. The method of calculation of absolute units from this type of measurement has been described by Kamen (4). The activities of all samples were measured with a standard deviation of 2 to 5% of the net counting rate (4).

The Extraction of Acid-soluble Phosphorus

The methods used in the present work for the extraction from tissues of the acid-soluble phosphorus fraction were based on those described by Le Page and Umbreit (6). The following procedure was carried out in a room the temperature of which was 5° to 8° C. The frozen tissue was ground to a powder with a cold stainless steel mortar and pestle. The powdered tissue was transferred while still frozen to a test tube containing 10% trichloroacetic acid. Approximately 5 ml. of 10% trichloroacetic acid were used for small organs such as the prostate gland. Larger volumes (up to 20 ml.) were used when larger organs such as the liver were to be analyzed. The weight of tissue was obtained by weighing the tube and its contents before and after the addition of the frozen powder. The tissue was then ground in the tube by the procedure of Potter and Elvehjem (7) using a pestle made of Plexiglass. The resulting homogenate was transferred to a volumetric flask of suitable size using 10% trichloroacetic acid. The concentration of tissue in the homogenate was such that 10.0 ml. (the volume usually fractionated) contained 0.07 to 0.6 gm. of tissue, depending on the weight of the tissue that was ground. Aliquot parts of the homogenate were taken for the determination of the total P^{31} and total P^{32} contents of the tissues.

Two 10.0 ml. volumes of the homogenate were measured into separate 40 ml. cone-shaped, heavy-wall Pyrex centrifuge tubes and each was treated in the following manner. The tube was centrifuged and the supernatant solution was transferred to a 50 ml. volumetric flask. The residue was washed three times by suspending it in 10-ml. portions of 10% trichloroacetic acid and centrifuging. The supernatant solutions were combined and the volume was made up to 50 ml. with 10% trichloroacetic acid. The residue was set aside for the extraction of phosphorus soluble in alcohol–ether. Duplicate

* *Model 161, Instrument Development Co. Ltd., Chicago, Ill.*

aliquot portions of the trichloroacetic acid extract were taken for the measurement of its content of P^{31} and P^{32}. Other aliquot portions were used for the determination of P^{31} present as inorganic phosphate. The amount of P^{31} present as organic acid-soluble P^{31} was determined by taking the difference between the values obtained for total acid-soluble P^{31} and inorganic acid-soluble P^{31}. Inorganic phosphate was precipitated from the trichloroacetic acid extract by the method of Delory (2) after the addition of 50 μgm. of P^{31} as inorganic phosphate to serve as a "carrier" of radioactive phosphate. The precipitate was dissolved in 1 ml. of 72% perchloric acid and the P^{32} content of the solution was determined. The supernatant and wash liquids were digested with nitric and perchloric acids in order to determine the amount of P^{32} present as organic acid-soluble P^{32}.

It was established by experiment that no appreciable amount of phosphorus was contained in the last of four successive trichloroacetic acid extracts of a sample of tissue. Approximately 0.5 gm. of rat liver was extracted with four 10 ml. portions of 10% trichloroacetic acid and each extract was analyzed separately. The data are recorded in Table I.

TABLE I

THE AMOUNTS OF PHOSPHORUS IN SUCCESSIVE TRICHLOROACETIC ACID EXTRACTS OF APPROXIMATELY 0.5 GM. OF RAT LIVER

Number of extract	P^{31} present in extract, mgm.	
	Experiment 1	Experiment 2
1	0.415	0.402
2	0.017	0.016
3	0.014	0.016
4	0.006	0.008

THE EXTRACTION OF ALCOHOL-ETHER-SOLUBLE PHOSPHORUS

The residue obtained after the removal of acid-soluble phosphorus was extracted with ethanol by suspending it and centrifuging it twice with 5-ml. portions of 95% ethanol. The supernatant solutions were removed to a 100 ml. Kjeldahl flask. Ten milliliters of a mixture of ethanol and ether (three volumes of 95% ethanol and one volume of diethyl ether) was than measured into the tube, a Pyrex glass bead was added, a small cold-finger condenser was inserted into the mouth of the tube, and the mixture was boiled under reflux for one-half hour. At the end of this time the tube was centrifuged and the supernatant solution was combined with the ethanol extracts. The extraction with 10 ml. of ethanol–ether was performed three times. After pouring off the fluid from the last extraction the tubes were placed, unstoppered, in a warm water bath until the contents were dry. The contents of the Kjeldahl flask were evaporated to a small volume over

gentle heat, about 5 ml. of water was added, and the contents of the flask were again evaporated to a small volume. Finally, concentrated nitric acid was added and the analysis of the extract was completed as described above.

The above extraction procedure was developed as the result of experiment. Approximately 0.5 gm. of rat liver was extracted with 10% trichloroacetic acid and the residue was extracted with ethanol and with ethanol–ether as described above. Each extract was analyzed separately. The results of the experiment are recorded in Table II.

TABLE II

THE AMOUNTS OF PHOSPHORUS PRESENT IN SUCCESSIVE ETHANOL AND ETHANOL-ETHER EXTRACTS OF APPROXIMATELY 0.5 GM. OF RAT LIVER

Solvent	Number of extract	P^{31} found, mgm.	
		Experiment 1	Experiment 2
Ethanol, 95%	1	0.212	0.278
	2	0.161	0.131
Ethanol–ether, 3 : 1	1	0.089	0.091
	2	0.014	0.012
	3	0.006	0.006
	4	0.002	0.003

In another experiment the period of extraction with boiling ethanol–ether was two hours instead of one-half hour. No more phosphorus was extracted by boiling for the longer time than for the shorter. The results of this experiment are recorded in Table III.

TABLE III

A COMPARISON OF THE AMOUNTS OF PHOSPHORUS EXTRACTED FROM SAMPLES OF RAT TISSUES BY BOILING WITH 3 : 1 ETHANOL–ETHER FOR DIFFERENT PERIODS OF TIME

Tissue	Ethanol-ether-soluble P^{31}, mgm./gm. fresh wt. of tissue	
	One-half hour	Two hours
Liver	1.07	1.08
Thymus	0.54	0.52

THE SEPARATION OF RIBONUCLEOTIDE PHOSPHORUS AND THYMONUCLEOTIDE PHOSPHORUS

For the separation of the ribonucleotide phosphorus and thymonucleotide phosphorus the method of Schmidt and Thannhauser (8) was employed. The method was modified by the use of 1 *N* sodium hydroxide instead of

1 *N* potassium hydroxide. This change was introduced in order to avoid the precipitation of potassium perchlorate during the oxidation of the organic material with perchloric acid.

Steudel and Peiser (10) found that ribonucleic acid is quantitatively split into acid-soluble nucleotides when treated with approximately 3% sodium hydroxide at room temperature for 24 hr. In the present work it was found that 97.5% of the phosphorus of purified calf thymus nucleic acid* was insoluble in 5% trichloroacetic acid after 17 hr. incubation at 35° C. in 1 *N* sodium hydroxide and that 96.5% of the phosphorus of purified sheep liver ribonucleic acid became soluble under the same conditions.

The procedure used for the separation of ribonucleotide phosphorus and thymonucleotide phosphorus was as follows. To the centrifuge tube containing the dried residue after extraction of the alcohol-ether-soluble phosphorus, 1 *N* sodium hydroxide (10 ml. per gm. fresh weight of tissue) was added. The tube was stoppered and placed in an incubator at 35° C. where it was left overnight. The next morning the solution was transferred quantitatively to a suitable volumetric flask using 1 *N* sodium hydroxide to wash the tube and to fill the flask to the mark. Duplicate aliquot portions of the solution were used for the determination of their contents of P^{31} and P^{32}.

To other duplicate aliquot parts of the solution were added 0.2 volume (of the aliquot) of 6 *N* hydrochloric acid and one volume (of the aliquot) of 5% trichloroacetic acid. The precipitate was separated by centrifugation and washed twice with 5-ml. portions of 2.5% trichloroacetic acid. The supernatant and wash liquids (containing ribonucleotide phosphorus) were combined and analyzed for their contents of P^{31} and P^{32}. The precipitate (containing thymonucleotide phosphorus) was also analyzed for its contents of P^{31} and P^{32}.

Application of the Fractionation Procedure

An experiment was performed to test the ability of the above procedures to permit the quantitative separation of phosphorus-containing substances added to a sample of tissue. The liver of a rat was homogenized in 10% trichloroacetic acid and the homogenate was diluted to a suitable volume. Duplicate aliquot portions of the homogenate were fractionated and the P^{31} contents of the fractions were determined by the procedures described above.

To another aliquot part of the same homogenate were added known amounts of phosphorus in the forms of a solution of potassium dihydrogen phosphate, a solution of disodium phenyl phosphate, a fine suspension of purified sheep liver ribonucleic acid, and a solution of purified calf thymus nucleic acid. The mixture was homogenized, made to suitable volume in 10% trichloroacetic acid and duplicate aliquot portions of the homogenate were fractionated and analyzed as described above. From the results of the analyses of the

* *The authors wish to thank Dr. G. C. Butler for the specimens of purified calf thymus nucleic acid and sheep liver ribonucelic acid used in this investigation.*

fractions of the tissue before and after the addition of the phosphorus-containing substances, the recovery of the phosphorus of the added substances was calculated. The results of this experiment are recorded in Table IV. From

TABLE IV

THE RECOVERY OF KNOWN AMOUNTS OF PHOSPHORUS ADDED AS POTASSIUM DIHYDROGEN PHOSPHATE, DISODIUM PHENYL PHOSPHATE, PURIFIED SHEEP LIVER RIBONUCLEIC ACID, AND CALF THYMUS NUCLEIC ACID TO A HOMOGENATE OF RAT LIVER IN TRICHLOROACETIC ACID

Fraction	Amount of P^{31} added, mgm.	Amount of P^{31} recovered, mgm.
Inorganic phosphate P^{31}	0.20	0.22
Acid-sol. organic P^{31}*	0.28	0.24
Alcohol-ether-sol. P^{31}	0.00	0.06
Ribonucleotide P^{31}	0.36	0.32
Thymonucleotide P^{31}	0.24	0.21

**Difference between total acid-soluble P^{31} and inorganic acid-soluble P^{31}.*

these data it was concluded that it was possible, by application of the procedures described in this communication, to account satisfactorily for the phosphorus of added substances in the appropriate fractions.

THE POSSIBILITY OF EXCHANGE OF P^{32} BETWEEN FRACTIONS

Before the procedures described herein could be used to determine the distribution of P^{32} among the phosphorus-containing constituents of tissues, it was necessary to determine to what extent exchange of P^{32} took place between the various fractions while they were being separated.

The liver of a rat was homogenized in 10% trichloroacetic acid at room temperature in the presence of a known amount of P^{32} as inorganic phosphate. As soon as possible after diluting the homogenate to a suitable volume in a volumetric flask duplicate aliquot portions were taken for fractionation and analysis by the above procedures. The homogenate was stored in the refrigerator and other samples were removed for analysis 24 and 48 hr. later. The results of these analyses are recorded in Table V.

The data of Table V show that the P^{32} added to the homogenate as inorganic phosphate remained almost quantitatively in the acid-soluble phosphorus fraction. About 10% of the P^{32} was present in the organic acid-soluble phosphorus fraction as a result of exchange of inorganic phosphate with esterified phosphate or as a result of failure to effect a quantitative precipitation of inorganic phosphate by the method of Delory.

It was found possible to decrease the amount of P^{32} that entered the organic acid-soluble phosphorus fraction by carrying on all operations at a temperature of 5° to 8° C. and by working quickly. When this was done the inorganic

TABLE V

THE EXCHANGE OF P^{32} ADDED AS INORGANIC PHOSPHATE TO A HOMOGENATE OF RAT LIVER IN TRICHLOROACETIC ACID

The extraction of acid soluble phosphorus was performed at room temperature. The amount of P^{32} present in a given fraction is expressed in terms of a percentage of the amount of P^{32} present in the homogenate

Fraction	Time at which fractionation started:		
	Start of expt.	24 hr.	48 hr.
Homogenate	100	100	100
Total acid-sol. P	96.5	93.0	94.7
Inorganic phosphate	85.5	84.7	84.2
Organic acid-sol. P	11.0	8.3	10.5
Alcohol-ether-sol. P	0.002	0.002	0.002
Extracted residue	0.004	0.004	0.005
Ribonucleotide P	0.004	0.003	0.004
Thymonucleotide P	0.001	0.001	0.002

phosphate fraction was found to contain 101.0% and the organic acid-soluble phosphorus fraction 3.4% of the total P^{32} present in the homogenate. In a similar experiment the corresponding values obtained were 99% and 2.8% respectively. These data were taken as an indication of the absence of an appreciable amount of exchange under the conditions of the experiment and established the quantitative nature of the precipitation of inorganic phosphate from a trichloroacetic acid extract by the method of Delory.

APPLICATION OF THE PROCEDURE TO EXPERIMENTS WITH THE RAT

In order to illustrate the application of the procedures reported in the present paper the following typical experiment is described.

A single male hooded rat, three months old and of 279 gm. body weight was used in this experiment. It received, by intraperitoneal injection from a calibrated hypodermic syringe and without being anesthetized, approximately 180 μc. of P^{32} as phosphate in a neutral physiological saline solution. The animal was placed immediately in a cage that permitted the separate collection of urine and feces. Water and food (Purina Fox Chow Checkers) were provided *ad libitum*.

Twenty-four hours after the injection of the solution containing P^{32} the rat was killed in the following manner. Ten minutes before the expiration of the 24 hr. experimental period the animal was held by hand in the metabolism cage and was anesthetized by the intraperitoneal injection of a solution of sodium pentobarbital (50 mgm. per kgm. of body weight). As soon as the animal became unconscious (usually about five minutes after the injection) 0.10 ml. of a solution of heparin (1000 units per ml., Connaught Laboratories, Toronto) was injected into one of the external jugular veins and one minute

later 1 ml. of blood was withdrawn from this vein using a different syringe. The animal was then frozen by pouring liquid nitrogen over it. The body of the rat was allowed to soften, but not to thaw in a room of which the temperature was 5° to 8° C. and was placed on a large watch glass for dissection. The liver, spleen, both kidneys, both testes, prostate gland, thymus, muscle from hind legs, and lymph nodes from the neck, axillae, and peritoneal cavity were removed. As soon as the tissues were excised they were placed in tightly stoppered glass weighing bottles and stored in the freezing compartment of a refrigerator. The remainder of the rat was placed in a 3 liter beaker. The instruments and watch glass used for the dissection were washed with water that was collected in the beaker. The amounts of P^{31} and P^{32} present in the contents of the beaker were determined.

The feces were collected from the metabolism cage and the cage was washed with several small volumes of water. The washings were combined with the urine and the P^{31} and P^{32} contents of the urine and feces were determined.

Each tissue was frozen with liquid nitrogen, and powdered with a stainless steel mortar and pestle, both of which were kept cold with liquid nitrogen. The ground frozen tissue was homogenized in trichloroacetic acid, was subjected to the fractionation procedures described above, and the P^{31} and P^{32} contents of the fractions were determined. The results of this experiment are given in Tables VI and VII.

The data of Table VI show that the sums of the amounts of P^{31} found in the individual fractions of the tissues are always within 15% and usually within 10% of the amounts found in the complete tissue. The sums of the amounts of P^{32} found in the individual fractions were always within 12% and usually within 7% of the amounts of P^{32} found in the complete tissue.

The data of Table VII show that a quantitative recovery of the injected P^{32} was made by analysis of all of the parts of the animal.

Discussion

The methods described in this communication provide a means of determining the distribution of P^{32} among the phosphorus-containing constituents of tissues following the administration of radioactive phosphate to the rat. The phosphorus-containing constituents were separated into groups that may be further fractionated and that are relatively well characterized.

By grinding the tissues in trichloroacetic acid and by fractionating aliquot portions of the resulting homogenate it was possible to obtain accurate sampling of the tissue and good agreement between the results of analyses of corresponding fractions of duplicate aliquots. The use of small amounts of tissues (0.07 to 0.06 gm. fresh weight of tissue) made it possible to perform the fractionation in a single tube thus avoiding losses that otherwise might have occurred through transfer.

TABLE VI

THE DISTRIBUTION OF P^{31} AND P^{32} AMONG PHOSPHORUS-CONTAINING FRACTIONS OF TISSUES OF A RAT 24 HR. AFTER THE INTRAPERITONEAL INJECTION OF A SOLUTION CONTAINING APPROXIMATELY 180 μC. OF P^{32} AS INORGANIC PHOSPHATE

P^{31} data are expressed as mgm. P^{31} per gm. fresh weight of tissue. P^{32} data are expressed as percentage of the dose of P^{32} administered found per gm. fresh weight of tissue

Fraction	Liver		Kidney		Spleen		Testis		Lymph node		Thymus		Prostate gland		Muscle	
	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}	P^{31}	P^{32}
Complete homogenate	3.28	0.44	2.76	0.32	2.22	0.30	2.18	0.069	3.4	0.34	4.4	0.65	2.25	0.21	2.47	0.15
A. Total acid-sol. P	1.08	0.18	0.79	0.16	0.82	0.15	0.74	0.052	0.71	0.21	1.06	0.21	0.60	0.091	2.2	0.15
Inorganic phosphate	0.51	0.057	0.49	0.069	0.30	0.056	0.52	0.025	0.41	0.069	0.43	0.078	0.34	0.048	0.72	0.063
Organic acid-sol.*	0.57	0.11	0.30	0.047	0.52	0.080	0.22	0.013	0.30	0.050	0.63	0.070	0.26	0.030	1.5	0.065
B. Alcohol-ether-sol. P	1.09	0.22	0.96	0.13	0.44	0.053	0.58	0.012	0.59	0.072	0.56	0.075	0.68	0.083	0.45	0.008
C. Extracted residue	0.9	0.039	0.72	0.033	0.97	0.072	0.69	0.006	2.28	0.095	3.2	0.39	1.3	0.023	0.21	0.002
Ribonucleotide P	0.77	0.045	0.51	0.036	0.57	0.052	0.53	0.006	1.33	0.066	1.81	0.26	0.88	0.038	0.18	0.002
Thymonucleotide P	0.16	—	0.18	—	0.39	0.024	0.11	—	0.95	0.020	1.70	0.16	0.17	—	0.04	—
Sum of A, B, C	3.07	0.44	2.47	0.32	2.23	0.27	2.01	0.070	3.58	0.38	4.8	0.68	2.58	0.20	2.86	0.16

* *Organic acid-sol. P^{31} was measured by subtracting inorganic acid-sol. P^{31} from total acid-sol. P^{31}. Organic acid-sol. P^{32} was measured directly.*

TABLE VII

THE DISTRIBUTION OF A KNOWN AMOUNT OF P^{32} IN THE RAT 24 HR. AFTER THE INTRAPERITONEAL INJECTION OF A SOLUTION OF P^{32} AS PHOSPHATE

The values for the amount of P^{32} found in the tissues are given in terms of percentage of the dose of P^{32} administered to the rat

Tissue analyzed	Amt. of P^{32} in tissue removed from rat, % of dose P^{32}	Tissue analyzed	Amt. of P^{32} in tissue removed from rat, % of dose P^{32}
Liver	5.69	Bone	2.26
Kidneys	0.72	Blood	0.14
Spleen	0.32	Urine	18.3
Testes	0.22	Feces	22.0
Lymph nodes	0.09	Remainder of rat	52.9
Thymus	0.23		
Prostate gland	0.04	Sum of above	103.3
Muscle	0.38		

By determining P^{31} and P^{32} in the same solution the specific activity of the solution may be measured directly. This measurement is uninfluenced by errors in the weight of the material analyzed and its accuracy depends only on the accuracy with which P^{31} and P^{32} are determined.

The specific activity of the phosphorus of an individual fraction may be influenced by the extent to which this fraction has been prepared free of others and the extent to which exchange of phosphate between the fractions has been eliminated. The data of Table IV provide information concerning the completeness of separation of the fractions from one another. The data of Table V show that little or no exchange of phosphate occurred between inorganic phosphate and alcohol-ether-soluble phosphorus, ribonucleotide phosphorus, and thymonucleotide phosphorus. If the reasonable assumption is made that any exchange of phosphate between these fractions in a trichloroacetic acid medium takes place through the intermediate formation of inorganic phosphate, the data of Table V indicate that the extent of this exchange is negligible.

Acknowledgment

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ASSESSMENT OF HOSPITAL DIETS[1]

By G. Hunter[2], J. Kastelic[3], and M. Ball[4]

Abstract

Analytical values are given for vitamin A, ascorbic acid, thiamine, riboflavin, niacin, calcium, phosphorus, iron, and protein on 12 diets commonly used in a modern hospital. The foods were analyzed as served to the patient. Assessed on the basis of commonly accepted standards the diets are found to be deficient in various respects, and particularly in the B-vitamins, ascorbic acid, and iron.

Introduction

In view of the valuable findings of the R.C.A.F. nutritional laboratories respecting the diets of air force personnel during the war, at the end of the war, when No. 4 R.C.A.F. Nutritional Laboratory was turned over to the University here, it was thought that an investigation of hospital diets might be of some value in the many-sided problem of convalescence and rehabilitation. Accordingly, with the assurance of the co-operation of an Edmonton hospital, an investigation was undertaken to ascertain the nutritional adequacy of 12 hospital diets, including seven separate Sippy Diets.

All diets were collected at the hospital by a trained dietitian and assayed in the laboratory for vitamin A and carotene, thiamine, riboflavin, niacin, ascorbic acid, calcium, phosphorus, iron, and protein. The Progressive Sippy Diets and the Tube Fed Diet were one day collections. Longer collection periods were not necessary because of the constant and uniform composition of these diets. The Soft and Clear Fluid Diets were collected over a three day period. All other diets were collected over a five day period.

Collection of Samples

At breakfast time when about half the ward trays had been prepared in the hospital diet kitchen one tray was set aside as our sample meal.

From this tray the individual food items were weighed and transferred to a four liter brown screw top bottle—Bottle I—containing 15 ml. glacial acetic acid. The procedure was the same at lunch and at supper. The whole day's food, including between-meal snacks, was thus collected in Bottle I.

For purposes of daily assay additional corresponding samples of vitamin C containing foods were collected over the day in a separate bottle—Bottle II—in a volume of 5% metaphosphoric acid corresponding to the weights of the items.

It should be noted that shells were removed from eggs, stones from stone fruits, the bone from meat, and the skins from oranges or grapefruit before weights were taken and food placed in the bottle. Beets were not collected

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because their pigment interferes in colorimetric procedures used in the assay of some of the food constituents. When choices of beverages were permitted at the same meal a compromise was made. If milk was included in the sample meal, then in the next meal only the milk or cream in the tea or coffee was collected.

Bottles I and II were taken at the end of the day to the nutritional laboratory. Appendix I shows the amounts of the food items in the diets.

Preparation of Samples for Analysis

The day's food in Bottle I was mixed in batches in a Waring blendor. Enough water, in measured amount, was added when necessary for mixing. Besides the total weight of the food, the total volume of the mixed day's food was recorded. One-tenth of each day's mixed food was transferred to a brown glass bottle, stored in a refrigerator. The five day aliquots were thoroughly mixed.

All analyses, excepting those for ascorbic acid, were carried out on aliquots from this material.

The contents of Bottle II were homogenized in a Waring blendor, and determinations were carried out on each day's sample. The five days' values were then averaged.

Assay Methods

In general the methods used by the R.C.A.F. were followed.

Thiamine was determined by the fermentation method of Schultz, Atkin, and Frey (15).

Riboflavin was determined by the microbiological method of Snell and Strong (16).

Niacin was determined by the chemical method of Melnick, Oser, and Siegel (11), except in the case of the Sippy Diets where it was determined by the microbiological method described by Snell and Wright (17) and by Kiehl, Strong, and Elvehjem (9).

A comparison of the values for niacin found by the two methods is shown for six diets, as follows:

Diet	Method of assay	
	Microbiological	Chemical
	Niacin, μgm./100 ml. of food suspension	
Low Fat	315	381
Low Salt	264	306
Child Adm. Diabetic	450	420
Full Fluid	210	296
Tube Feeding	880	712
Sippy 1 to 5 days	69	140
Sippy 6th day	66	850
Sippy 7 to 8 days	56	400

The *vitamin A plus carotene* was determined by the Olcott and McCamm (13) modification of the Carr–Price method.

Ascorbic acid was determined by the usual method of titration with 2,6 : dichlorophenolindophenol, on Bottle II collections. Various checks were made for non-vitamin C reductants (18), but the amounts found were insignificant.

Protein—total nitrogen was determined by the Kjeldahl method with a mixture of mercuric oxide (HgO) and powdered selenium (14) with the usual conversion factor 6.25.

As a check on the Sippy Diet results, protein was determined in commercial milks as shown below by the standard method of nitrogen described under (1).

Grade A milk (3.5% fat), summer 1946		Cream—10% fat, summer 1946		Milk from hospital kitchen, winter 1945–46	
Bottle	Protein, %	Bottle	Protein, %	Sample	Protein, %
1	3.13	1	2.76	1	3.21
2	3.18	2	2.75	2	3.18
3	3.09	3	2.78	3	3.23

Calcium was determined by the method of Kramer and Tisdall (10).

Iron was determined by the method of Jackson (8).

Phosphorus was determined by the method of Fiske and Subbarow (4).

Check on Added Vitamins

The Tube Fed Diet was supplemented by certain vitamin preparations indicated in the table below. It is perhaps of some interest to compare the values found by our analytical methods with those calculated for milk, milk powder, and eggs from food tables and those for the vitamin preparations supplied to us by the Division of Pharmacy of the Hospital.

Food items in diet	Vit. A, I.U.	Thiamine, mgm.	Riboflavin, mgm.	Niacin, mgm.	Ascorbic acid, mgm.
Percomorph	10,000				
Betalin		10.00			
B. Plex		1.80	3.55	17.70	
Ascorbic acid					100.0
Milk, 1900 ml.	3200	0.80	3.40	2.10	
Milk powder, 100 gm.	1000	0.34	1.93	1.10	12.0
Eggs, 5	2300	0.30	0.82	0.15	
Total, calculated	16,500	13.24	9.70	21.05	112.0
Total, found	18,200	11.31	9.10	22.88	28.0

It will be thus seen that the only serious discrepancy is in the ascorbic acid values. It appears probable here that the amount of metaphosphoric acid used was insufficient to acidify the mixture and a large amount of the ascorbic acid was oxidized before titration was carried out.

Discussion of Results

All analytical values for the average day's intake are assembled in the chart (Fig. 1). As a guide to dietary energy supply calculated values for calories are included. As a basis for assessment of adequacy of dietary constituents, the Recommended Dietary Allowances of the Food and Nutrition Board, National Research Council, U.S.A. (12) are indicated, as the averages for sedentary men and sedentary women, by hatched bars.

We shall briefly comment* on the diets as illustrated in Fig. 1.

Comments on Diets

Full House.—This is the general diet for the Hospital and its general adequacy should be of some concern to us. Judged on the basis of Recommended Dietary Allowances it is adequate in protein, calcium, phosphorus, iron, and vitamin A, but inadequate in calories, vitamin C, and the B-vitamins, thiamine, riboflavin, and niacin. Its energy value should be increased by about 20%, its vitamin C and riboflavin doubled, its niacin tripled, and its thiamine increased fourfold.

Sippy Diets.—During the first two weeks the diets present a regime of definite insufficiency upon which only a small patient would not lose weight. Throughout the three weeks the diets are adequate only in calcium, phosphorus, and riboflavin. The vitamin A should be doubled and the iron and thiamine should be tripled on the average over the three week period. With respect to ascorbic acid and niacin the diet appears well designed to produce both scurvy and pellagra.

Initial Postulcer.—This diet is adequate in most respects. Its iron should be increased 50% and its niacin at least doubled. It is also barely adequate in thiamine and riboflavin.

Admission Diabetic.—The calorie values would here appear about 25% too low. The iron, riboflavin, and niacin should be increased by 50% and the thiamine increased two to four times. Vitamin C is here adequate.

Low Residue.—The amounts of iron, ascorbic acid, thiamine, and niacin present in this diet should all be about doubled. The diet is in other respects adequate.

Low Salt.—The B-vitamins here should be increased by 50%.

* *In this connection we should like to note that our comments should not be interpreted in a mechanical fashion. We are aware of the inadequacy of comparing the diets of hospital patients with a very tentative standard for healthy people, but above all we do not want to imply that poor diets should be mechanically supplemented by synthetic vitamins or mineral preparations. The omments are made merely as a guide to the improvement of the whole food diets.*

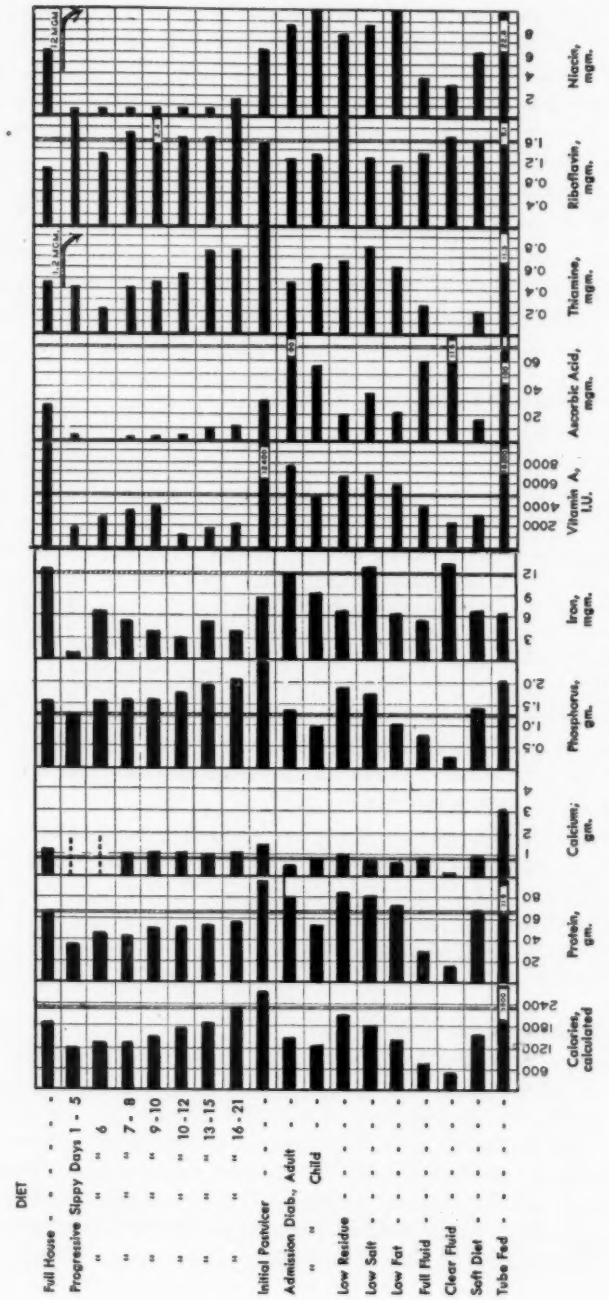

FIG. 1. *Hospital diets: daily constituent intake.*

Low Fat.—The calorie value of this diet is 50% too low. Its iron, ascorbic acid, and each of the three B-vitamins should be doubled.

Full Fluid.—These diets are used only over very short periods in acute conditions of the gastrointestinal tract and in association with operative intervention, so one need not be unduly concerned with their low energy values and other deficiencies.

Soft Diet.—This diet has only about 15% of the recommended thiamine allowance. It is also quite inadequate in iron, ascorbic acid, and niacin.

Tube Feeding.—With its high calorie value, high mineral and vitamin values this diet should quickly repair undernutrition. It is notable however that the diet contains only half the recommended iron allowance. The balance of added vitamins is open to question.

Comments on Dietary Constituents

With the exception of only four diets out of 18, the energy value of the diets supplied is appreciably lower than the recommended sedentary allowances.

In general, the protein, calcium, and phosphorus allowances of the patients are met, and sometimes more than met.

Iron is adequate in the Full House Diet, in the Adult Admission Diabetic, in the Low Salt, and in the Clear Fluid Diet. Iron is deficient in the Sippy Diets, in the Low Fat Diet, in the Soft Diet, and in the Tube Fed Diet.

Except for the Sippy Diet the vitamin A supplied is fairly adequate.

Ascorbic acid is at a satisfactory level in the Admission Diabetic Diets, in the Full Fluid Diet, in the Clear Fluid Diet, and in the Tube Fed Diet.

The B-vitamins, and especially thiamine and niacin, judged on the basis of recommended allowances, are alarmingly low. Special comment is called for respecting those two constituents.

It should first be noted that the "recommended allowance", with respect to vitamins, is assumed to be at least 50% greater than the minimal requirements for the prevention of definite signs of deficiency. The diets under study are so low in thiamine and niacin that the question arises as to whether they are meeting minimal requirements for the avoidance of beriberi and pellagra. The question can be answered more clearly for thiamine than for niacin.

Minimal Thiamine Requirements

If we calculate the vitamin/calorie index used by Cowgill (2) in his studies of the incidence of beriberi as observed up to 1934 we find for the daily intake of 0.43 mgm. of thiamine an index: 2276/1900 or 1.15. Of course our calorie value is an estimate and we do not have an average weight of patient upon which to apply this index precisely, but on Cowgill's standards such an index would just support a patient 40 kgm. or 88 lb. in weight. It is certain that the average weight of patients in the hospital is considerably above this figure, and as Cowgill found that with an index less than 1.76 there was an

appreciable incidence in the groups studied, so we might expect an appreciable incidence of beriberi in patients after perhaps a month or two on this thiamine intake.

The fact that beriberi does not exist under such circumstances in the hospital is an indication that the Cowgill standards are too high, or the patients' stay in hospital is too short for deficiency signs to appear.

A recent review on *The Thiamin Requirements of Man* by Holt (5) enables us to reach a more definite conclusion on the subject. Holt, on the basis of rigorous criteria, places the minimum requirement for the normal adult "between 0.17 and 0.23 mg. per 1000 calories", for the prevention of definite beriberi. On a mean value of 0.20, and assuming our calorie intake to be about 2000, the thiamine intake of 0.43 just exceeds the minimum. However Holt adds: "A range intake between 0.24 and 0.44 mg. per 1000 calories appears to be protective against thiamin deficiency."

If we take the mean value here of 0.34, then our House Diet should contain at least 0.6 mgm. of thiamine per day.

On the basis of such observations we conclude that the Full House Hospital Diet is at least 50% below conservative thiamine requirements.

Minimal Niacin Requirements

We have no reliable observations on the minimum requirements of niacin to prevent pellagra in humans. It has been calculated (see Dann (3)) that the pellagrous subjects of Goldberger and Wheeler ingested 7.2 mgm. daily. Winters and Leslie (see (3)) in a study of low income groups in Texas found by analysis a mean intake of 4.2 mgm. of niacin per day with no pellagra. Calculations from blacktongue tests on dogs to the 70 kgm. man indicate a requirement of 10 mgm., but this is not regarded as a valid inference as niacin metabolism in man and dog differ.

The Full House Diet containing 5.95 mgm. of niacin, on the basis of present knowledge, could not be regarded as generous. On the three weeks of the Sippy Diet the average daily intake is only 0.93 mgm. But because pellagra is not produced over this period one could not assume that the minimum requirement is less than 0.93 mgm.

The Full House Diet, and several of the others, but especially the Sippy Diets, provide inadequate amounts of niacin.

Results in Relation to Foods Supplied

The prevalence of milk in the diets assures a general adequacy of calcium and phosphorus and adds significantly to the protein and riboflavin. Milk however is low in iron and so there is an average daily intake over the Sippy period of only 4.4 mgm. The main food sources of iron are meats, breads, vegetables, and eggs. There is adequacy of iron in the Full House, Adult

Admission Diabetic, and Low Salt Diets from such sources. The high iron in the Clear Fluid Diet was mostly obtained from boiled down green vegetable juice.

The high values found in the Full House Diet and the Initial Postulcer Diet for vitamin A are to a large extent accounted for by carrots supplied in two or three meals out of the five day collection. This emphasizes how readily a poor nutritional picture may be changed to a good one.

Ascorbic acid is adequate only where citrus fruits have been provided. The non-citrus foods used by the hospital are apparently unsuitable to provide an adequate vitamin C intake. The titrations on the daily vitamin C food collections reflected this fact very strikingly.

The vitamin C should be increased in the Full House Diet, in the Sippy Diet, and in others by increasing the citrus fruits supplied, or by other means, such as preparations from wild rose hips, which are a far richer source of ascorbic acid and abundantly home grown. Failing natural sources synthetic ascorbic acid should be supplied.

In the Full House Diet it appears that about 20% of both the thiamine and niacin was derived from bread; on an average about 0.1 mgm. thiamine and 2.0 mgm. niacin per day. From the dietary constituents it can be calculated that there was present in the daily food about 4 oz. of white flour. Were the white flour replaced by an equal quantity of Canada Approved Flour, the daily diet would gain about 0.33 mgm. of thiamine and 4.1 mgm. niacin (6) thus taking the diet definitely out of the danger zone that exists for those two vitamins.

General

We have chosen the mean allowances of the U.S. National Research Council, for sedentary men and women, as a conservative basis on which to assess particularly the Full House Hospital Diet. On this basis it has been shown to be low in calories, in all water soluble vitamins, and even dangerously low in thiamine and niacin.

There is no scientific evidence to support the belief that large amounts of vitamins hasten healing and regenerative processes except where there has been previous deficiency. The average convalescent patient therefore needs no more than the ordinary sedentary individual. But, as proper nutrition is a fundamental basis of health, and patients are in hospital to regain health, the general adequacy of a hospital diet should not be open to question. Where there is faulty nutrition in the local population (7) as there is with respect especially to iron and the water soluble vitamins, it would appear that the hospital meals should be designed to counteract such conditions rather than perpetuate and even exacerbate them.

There would seem to be little justification for the continuation of the unsupplemented Sippy regime. It is definitely established that there is practically no wound healing in scurvy, and vitamin C has a structural function in collagen

formation. There is recent evidence that the daily requirement of vitamin C may be nearer 30 mgm. than 70 mgm. but the Sippy regime provides a daily average of only 4 mgm. The danger of surgical intervention on patients after prolonged periods on the Sippy regime would appear to be considerable.

It would be of little use, even if we were competent, to pursue this discussion into the adequacy of the special diets in meeting the needs for which they are used. The subject of nutritional needs in relation to clinical conditions is very wide and varied, and only the medical practitioner is in a position to judge wisely how his patient should be fed. The dietary analyses and discussion presented in this paper may be helpful to practitioners in making use of nutrition as a means towards the health of their patients.

Finally, it should be emphasized that the diets in the particular hospital here investigated are likely to be at least as good as in the average Canadian hospital.

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(For Appendix see next page.)

Appendix I

House Diet—Full (five day collection)
Nov. 19 to 23, 1945

Day 1—Nov. 19, 1945

Milk (3¼%)	240 gm.
Bread, white	21 "
Bread, rye	20 "
Roll, white	36 "
Butter	13 "
Soda crackers	8 "
Graham crackers	15 "
Cream of wheat	144 "
Egg, scrambled	116 "
Cocoa	167 "
*Baked beans with tomato sauce	128 "
*Cabbage salad	55 "
*Soup, vegetable	138 "
*Parsnips, steamed	92 "
*Potato, steamed	80 "
Roast beef	40 "
Gravy	20 "
Caramel pudding	120 "
*Crab apples, canned	126 "

Total weight = 1579 gm.
Total volume = 2000 ml.

*Analyzed for vitamin C
Total weight = 574 gm.
Total volume = 1080 ml.

Day 2—Nov. 20, 1945

Milk (3¼%)	395 gm.
Bread, brown	35 "
Bread, white	18 "
Roll, white	45 "
Butter	17 "
Soda crackers	9 "
Graham wafers	15 "
Rolled oats	125 "
Bacon	35 "
Cocoa	156 "
Macaroni and cheese	160 "
*Soup, vegetable	108 "
*Tomatoes, stewed	44 "
*Potato, mashed	96 "
*Carrots, raw, shredded	40 "
Liver, baked	52 "
*Fruit salad (diced fruit)	155 "
Cottage pudding with chocolate sauce	72 "

Total weight = 1577 gm.
Total volume = 2000 ml.

*Analyzed for vitamin C
Total weight = 420 gm.
Total volume = 880 ml.

House Diet—Full, cont'd.

Day 3—Nov. 21, 1945

Milk (3¼%)	428 gm.
Bread, brown	19 "
Bread, white	35 "
Roll, white	38 "
Butter	23 "
Soda crackers	9 "
Graham wafers	15 "
*Potato, mashed	85 "
Brex	132 "
Egg, soft cooked	48 "
Cocoa	215 "
*Soup, vegetable	136 "
*Cabbage, steamed	56 "
*Potato, scalloped	85 "
*Lettuce salad	65 "
Meat loaf	81 "
Gravy	52 "
Bologna	56 "
*Prunes, stewed	92 "
Ice cream (Neapolitan)	46 "

Total weight = 1716 gm.
Total volume = 2620 ml.

*Analyzed for vitamin C
Total weight = 1519 gm.
Total volume = 1000 ml.

Day 4—Nov. 22, 1945

Milk (3¼%)	361 gm.
Bread, brown	15 "
Bread, white	13 "
Roll, white	50 "
Butter	13 "
Soda crackers	9 "
Graham wafers	14 "
Cream of wheat	118 "
Egg, fried	46 "
Egg, devilled	50 "
*Soup, vegetable and macaroni	141 "
*Peas and carrots	73 "
*Cabbage and carrot salad	73 "
*Potato, steamed	87 "
*Potato, baked	100 "
Roast beef	64 "
Vanilla cream	114 "
Sugar cookies	32 "
Cocoa	158 "

Total weight = 1531 gm.
Total volume = 2520 ml.

*Analyzed for vitamin C
Total weight = 474 gm.
Total volume = 1000 ml.

Appendix I—*Cont'd.*

House Diet—Full, concl'd.

Day 5—Nov. 23, 1945

Milk	253 gm.
Cocoa	200 "
Bread, brown	34 "
Bread, white	23 "
Roll, white	40 "
Soda crackers	9 "
Graham wafers	15 "
Rolled oats	178 "
Egg, soft cooked, 1, edible portion (E.P)	47 "
*Soup, barley	168 "
*Tomatoes, stewed	130 "
*Potato, mashed	127 "
*Carrot sticks	20 "
Salmon, baked	100 "
Shepherd's pie	194 "
Jello	126 "
Spice cake with icing	61 "
Butter	20 "
(Beets)	(60) "
Total weight	= 1745 gm.
Total volume	= 2400 ml.

*Analyzed for vitamin C

Total weight = 445 gm.
Total volume = 900 ml.

Progressive Sippy (1 to 5 days) Oct. 1, 1945

Milk : cream 1 : 4	1106 gm.
Total volume	= 1106 ml.

(Milk 3.5%, cream 10% fat: 1:4)

Progressive Sippy (6th day) Oct. 9, 1945

Milk and cream	1106 gm.
Eggs, whole, 2, E.P	160 "
Total weight	= 1266 gm.
Total volume	= 1150 ml.

Progressive Sippy (7th and 8th days) Oct. 22, 1945

Milk and cream	1106 gm.
Eggs, whole, 2, E.P	116 "
Cereal (cream of wheat)	85 "
Total weight	= 1307 gm.
Total volume	= 1330 ml.

Progressive Sippy (9th and 10th days) Nov. 5, 1945

Milk and cream	1106 gm.
Eggs, whole, 2, E.P	130 "
Cereal (cream of wheat)	170 "
Cocoa	85 "
Total weight	= 1491 gm.
Total volume	= 1460 ml.

Progressive Sippy (11th and 12th days) Nov. 19, 1945

Milk and cream	1106 gm.
Eggs, whole, 2, E.P	104 "
Cereal (rolled oats)	85 "

Progressive Sippy, concl'd.

Cocoa	85 "
Jello (1 serving)	110 "
Caramel cream (1 serving)	115 "
Total weight	= 1605 gm.
Total volume	= 1760 ml.

Progressive Sippy (13th to 15th days) Nov. 19, 1945

Milk and cream	1106 gm.
Cereal (rolled oats)	85 "
Egg, whole, 1, E.P	64 "
Custard (1 serving)	122 "
Milk toast, white bread	36 "
milk	100 "
Jello (1 serving)	114 "
Potato, mashed (1 serving)	130 "
Butter	5 "
Total weight	= 1762 gm.
Total volume	= 1840 ml.

Progressive Sippy (16th to 21st days) Nov. 19, 1945

Milk and cream	1106 gm.
Cereal (rolled oats)	85 "
Toast (white), 2 slices	80 "
Eggs, whole, 2, E.P	110 "
Ice cream, vanilla (1 serving)	60 "
Caramel cream (1 serving)	106 "
Custard (1 serving)	128 "
Potato, mashed (1 serving)	90 "
Butter	22 "
Total weight	= 1787 gm.
Total volume	= 1900 ml.

Initial Postulcer Diet (five day collection) Nov. 19 to 24, 1945

Day 1—Nov. 19, 1945

Milk (3¼%)	461 gm.
Milk, "Superior", 10%	599 "
Bread, white	128 "
Butter	22 "
Eggs, 1, E. P	54 "
Egg, creamed	70 "
Cream of wheat	150 "
*Jam, peach	14 "
Custard	56 "
Caramel cream	96 "
*Soup, cream of tomato	237 "
*Peas, green	52 "
*Potato, baked, E.P	112 "
*Potato, steamed	92 "
Salmon, steamed	105 "
Total weight	= 2248 gm.
Total volume	= 2800 ml.

*Analyzed for vitamin C

Total weight = 507 gm.
Total volume = 750 ml.

Appendix I—*Cont'd.*

Initial Postulcer Diet, cont'd.

Day 2—Nov. 20, 1945

Milk (3¼%)	654 gm.
Milk, "Superior", 10%	625 "
Bread, white	91 "
Butter	18 "
Egg, 1, E.P.	48 "
Rolled oats	180 "
*Jam, peach	24 "
Applesauce	90 "
Cottage pudding with chocolate sauce	54 "
Cup cake (iced)	36 "
*Soup, cream of potato	182 "
*Tomato juice	100 "
*Potato, baked E.P.	45 "
*Potato, mashed	100 "
Cheese, soufflé	92 "
Macaroni and cheese	92 "
*Carrots, steamed	35 "

Total weight = 2466 gm.
Total volume = 2920 ml.

*Analyzed for vitamin C
Total weight = 486 gm.
Total volume = 950 ml.

Day 3—Nov. 21, 1945

Milk (3¼%)	650 gm.
Milk, "Superior", 10%	592 "
Bread, white	126 "
Butter	17 "
Egg, 1, E.P.	40 "
Egg, creamed	76 "
Cream of wheat	112 "
*Jam, peach	16 "
*Tomato juice	200 "
Ice cream with chocolate sauce	88 "
*Prunes, stewed	108 "
Cottage cheese	96 "
*Soup, cream of tomato	120 "
*Soup, cream of pea	130 "
*Potato, scalloped	118 "
*Potato, mashed	76 "
*Peas, green	48 "

Total weight = 2613 gm.
Total volume = 3080 ml.

*Analyzed for vitamin C
Total weight = 816 gm.
Total volume = 1250 ml.

Initial Postulcer Diet, cont'd.

Day 4—Nov. 22, 1945

Milk (3¼%)	523 gm.
Milk, "Superior", 10%	624 "
Bread, white	86 "
Butter	29 "
Eggs, 2, E.P.	95 "
Cream of wheat	150 "
*Prunes, stewed	70 "
Custard	116 "
Vanilla cream	122 "
Cottage cheese	88 "
*Soup, cream of tomato	143 "
*Soup, cream of pea	140 "
*Tomato juice	106 "
*Potato, baked E.P.	100 "
*Potato, steamed	88 "
*Carrots, steamed	52 "

Total weight = 2532 gm.
Total volume = 3315 ml.

*Analyzed for vitamin C
Total weight = 699 gm.
Total volume = 1130 ml.

Day 5—Nov. 23, 1945

Milk (3¼%)	600 gm.
Milk, "Superior", 10%	574 "
Bread, white	101 "
Butter	23 "
Egg, 1, E.P.	36 "
Rolled oats	143 "
*Tomato juice	93 "
*Jam, peach	13 "
Jello	156 "
*Fruit juice, mixed	110 "
Custard	100 "
Cottage cheese	68 "
*Soup, cream of potato	112 "
*Soup, cream of carrot	153 "
*Potato, mashed	106 "
*Potato, steamed	80 "
Salmon, steamed	66 "
(Beets)	(74) "

Total weight = 2534 gm.
Total volume = 3000 ml.

*Analyzed for vitamin C
Total weight = 667 gm.
Total volume = 1200 ml.

Appendix I—*Cont'd.*

Admission Diabetic Diet (five day collection) Jan. 15 to 20, 1946

Day 1—Jan. 15, 1946

Milk (3¼%)	240 gm.
Bread, brown	60 "
Butter	30 "
Rolled oats	180 "
Egg, soft cooked, 1, E.P.	55 "
Broth	160 "
Beef, cold, sliced	60 "
Beef, roast	60 "
*Potato, baked	50 "
*Potato, steamed	90 "
*Beans, yellow	100 "
*Peas, green	50 "
*Plums, greengage, canned	150 "
*Peaches, canned	150 "
*Grapefruit	150 "

Total weight = 1585 gm.
Total volume = 2520 ml.

*Analyzed for vitamin C
Total weight = 740 gm.
Total volume = 1250 ml.

Day 2—Jan. 16, 1946

Milk (3¼%)	240 gm.
Bread, brown	60 "
Butter	30 "
Brex	180 "
Egg, soft cooked, 1, E.P.	55 "
Broth	180 "
Beef, roast	60 "
Cheese, Canadian	40 "
Macaroni	60 "
*Potato, steamed	90 "
*Tomatoes, stewed	40 "
*Turnips, steamed	75 "
*Applesauce	150 "
*Pears, canned	150 "
*Cherries, Royal Anne, canned	150 "

Total weight = 1560 gm.
Total volume = 2540 ml.

*Analyzed for vitamin C
Total weight = 655 gm.
Total volume = 1100 ml.

Admission Diabetic Diet, cont'd.

Day 3—Jan. 17, 1946

Milk (3¼%)	240 gm.
Bread, brown	60 "
Butter	40 "
Cream of wheat	180 "
Egg, soft cooked, 1, E.P.	45 "
Broth	190 "
Beef, cold, sliced	90 "
Beef, ground	60 "
*Potato, steamed	140 "
*Tomatoes, stewed	60 "
Beets, steamed	75 "
*Lettuce	40 "
*Apricots, canned	150 "
*Fruit salad (cherries, peaches)	150 "
*Prunes, stewed	50 "
Roll, white	30 "
*Oranges, 2, E.P.	150 "

Total weight = 1750 gm.
Total volume = 3100 ml.

*Analyzed for vitamin C
Total weight = 740 gm.
Total volume = 1350 ml.

Day 4—Jan. 18, 1946

Milk (3¼%)	240 gm.
Bread, brown	90 "
Butter	60 "
Rolled oats	180 "
Egg, soft cooked, 1, E.P.	55 "
Broth	200 "
Salmon, steamed	60 "
Cottage cheese	60 "
Cheese, Canadian	20 "
*Potato, steamed	90 "
*Potato, baked	50 "
*Peas, green, canned	65 "
*Lettuce	20 "
*Carrots, grated	30 "
*Oranges, E.P.	225 "
*Pears, canned	150 "
*Plums, greengage, canned	150 "

Total weight = 1745 gm.
Total volume = 2360 ml.

*Analyzed for vitamin C
Total weight = 780 gm.
Total volume = 1320 ml.

Appendix I—*Cont'd.*

Admission Diabetic Diet, concl'd.

Day 5—Jan. 19, 1946

Milk (3¼%)	240 gm.
Bread, brown	60 "
Roll, white	40 "
Butter	50 "
Cream of wheat	180 "
Egg, soft cooked, 1, E.P.	45 "
Egg, hard cooked, 1, E.P.	30 "
Broth	220 "
Chicken, cold, sliced	60 "
Veal, roast	60 "
*Potato, steamed	90 "
*Potato, baked	50 "
*Beans, green, canned	75 "
*Turnip, steamed	75 "
*Peas, green, canned	35 "
*Applesauce	150 "
*Plums, greengage, canned	150 "
*Peaches, canned	150 "
*Oranges, E.P.	220 "

Total weight = 1980 gm.
Total volume = 3000 ml.

*Analyzed for vitamin C
Total weight = 995 gm.
Total volume = 1350 ml.

Child's Admission Diabetic Diet (five day collection) Feb. 18 to 22, 1946

Day 1, Feb. 18, 1946

Milk (3¼%)	540 gm.
Bread, brown	45 "
Butter	30 "
Soda crackers, 1	5 "
Cream of wheat	90 "
Broth	110 "
Beef, roast	30 "
Egg, soft cooked, 1, E.P.	45 "
Egg, poached	55 "
*Potato, steamed	60 "
*Spinach	100 "
*Beans, green	100 "
*Apricots, canned	100 "
*Applesauce	100 "
*Apple, 1, raw	100 "
*Grapefruit, half	105 "

Total weight = 1615 gm.
Total volume = 2660 ml.

*Analyzed for vitamin C
Total weight = 665 gm.
Total volume = 1190 ml.

Child's Admission Diabetic Diet, Cont'd.

Day 2—Feb. 19, 1946

Milk (3¼%)	540 gm.
Bread, brown	45 "
Butter	30 "
Soda crackers, 1	5 "
Brex	90 "
Broth	215 "
Beef, cold, sliced	30 "
Egg, soft cooked, 1, E.P.	45 "
Egg, poached	60 "
*Potato, steamed	60 "
*Beans, green	100 "
*Peas, green	35 "
*Tomato juice	100 "
*Cherries, Royal Anne, canned	100 "
*Apricots, canned	100 "
*Apple, 1, raw	200 "

Total weight = 1755 gm.
Total volume = 2450 ml.

*Analyzed for vitamin C
Total weight = 695 gm.
Total volume = 1200 ml.

Day 3, Feb. 20, 1946

Milk	540 gm.
Bread, brown	45 "
Butter	30 "
Soda crackers, 1	5 "
Rolled oats	90 "
Broth	205 "
Beef, roast	30 "
Cheese, Canadian	20 "
Egg, soft cooked, 1, E.P.	45 "
*Potato, steamed	60 "
*Peas, green	35 "
*Beans, green	30 "
*Celery, raw	20 "
*Lettuce	30 "
*Tomato slices	20 "
*Tomato juice	100 "
*Orange, 1	75 "
*Peaches, canned	100 "
*Pears, canned	100 "

Total weight = 1580 gm.
Total volume = 2000 ml.

*Analyzed for vitamin C
Total weight = 570 gm.
Total volume = 1050 ml.

Appendix I—*Cont'd.*

Child's Admission Diabetic Diet, cont'd.

Day 4, Feb. 21, 1946

Milk (3¼%)	540 gm.
Bread, brown	45 "
Butter	35 "
Soda crackers, 1	5 "
Cream of wheat	90 "
Broth	205 "
Lamb, cold, sliced	30 "
Cottage cheese	30 "
Egg, soft cooked, 1, E.P.	55 "
*Potato, steamed	60 "
*Carrots	50 "
*Beans, wax	50 "
*Turnip sticks	30 "
*Tomato slices	50 "
*Celery, raw	20 "
*Apple juice	100 "
*Orange, 1	80 "
*Plums, greengage	100 "
*Pears, canned	100 "

Total weight = 1675 gm.
Total volume = 2000 ml.

*Analyzed for vitamin C
Total weight = 640 gm.
Total volume = 1100 ml.

Day 5, Feb. 22, 1946

Milk (3¼%)	540 gm.
Bread, brown	45 "
Butter	40 "
Soda crackers, 1	5 "
Cream of wheat	90 "
Broth	235 "
Salmon, steamed	30 "
Cottage cheese	30 "
Egg, soft cooked, 1	55 "
*Potato, steamed	60 "
*Peas	35 "
*Tomatoes, canned	100 "
*Applesauce	100 "
*Orange, 1	80 "
*Peaches, canned	100 "
*Apple juice	100 "

Total weight = 1645 gm.
Total volume = 1500 ml.

*Analyzed for vitamin C
Total weight = 575 gm.
Total volume = 1000 ml.

Low Residue Diet—(five day collection) Jan. 15 to 19, 1946

Day 1—Jan. 15, 1946

Milk (3¼%)	560 gm.
Bread, white	100 "
Butter	30 "
Milk, "Superior", 10%	115 "
Egg, soft cooked, 1, E.P.	50 "
Egg, creamed	90 "
*Soup, cream of tomato	170 "
Salmon loaf	60 "
*Potato, mashed	135 "
*Potato, baked	80 "
Rolled oats	125 "
*Plum juice	175 "
*Jam, peach	10 "
Custard	95 "
Ice cream, vanilla	55 "

Total weight = 1850 gm.
Total volume = 2560 ml.

*Analyzed for vitamin C
Total weight = 570 gm.
Total volume = 1000 ml.

Day 2—Jan. 16, 1946

Milk (3¼%)	495 gm.
Bread, white	135 "
Butter	30 "
Milk, "Superior", 10%	60 "
Cream of wheat	130 "
Egg, soft cooked, 1, E.P.	50 "
*Soup, cream of carrot	125 "
Soup, tomato and beef	100 "
Cheese, creamed	95 "
Macaroni and cheese	200 "
*Potato, mashed	80 "
*Potato, creamed	65 "
*Tomato juice	185 "
*Jam, peach	10 "
*Applesauce, purée	80 "
Custard	160 "

Total weight = 2000 gm.
Total volume = 2820 ml.

*Analyzed for vitamin C
Total weight = 545 gm.
Total volume = 1050 ml.

Appendix I—*Cont'd.*

Low Residue Diet, cont'd.

Day 3—Jan. 17, 1946

Milk (3½%)	480 gm.
Bread, white	70 "
Butter	30 "
Milk, "Superior", 10%	65 "
Cream of wheat	150 "
Egg, soft cooked, 1, E.P.	55 "
Egg, creamed	80 "
*Soup, cream of tomato	200 "
Chicken, cold, sliced	35 "
*Potato, mashed	190 "
*Tomato juice	50 "
*Jam, peach	15 "
Custard	95 "
Cottage pudding	120 "
Roll, white	40 "

Total weight = 1675 gm.
Total volume = 2510 ml.

*Analyzed for vitamin C
Total weight = 455 gm.
Total volume = 1000 ml.

Day 4—Jan. 18, 1946

Milk (3½%)	520 gm.
Bread, white	90 "
Butter	30 "
Milk, "Superior", 10%	50 "
Rolled oats	165 "
Egg, soft cooked, 1, E.P.	50 "
Soup, cream of pea and beet	90 "
Soup, cream of carrot (strained)	110 "
Cottage, cheese	95 "
Salmon, steamed	75 "
*Potato, mashed	100 "
*Potato, scalloped	120 "
*Tomato juice	95 "
*Orange juice	90 "
*Jello, lemon	75 "
Custard	100 "
*Jam, peach	15 "

Total weight = 1870 gm.
Total volume = 2200 ml.

*Analyzed for vitamin C
Total weight = 495 gm.
Total volume = 1050 ml.

Low Residue Diet, concl'd.

Day 5—Jan. 19, 1946

Milk (3½%)	365 gm.
Bread, white	70 "
Butter	30 "
Milk, "Superior", 10%	100 "
Cream of wheat	150 "
Egg, soft cooked, 1, E.P.	45 "
*Soup, cream of pea purée	240 "
Egg, creamed	150 "
Salmon, creamed	50 "
*Potato, mashed	70 "
*Potato, baked	90 "
*Tomato juice	240 "
Chicken, cold, sliced	45 "
Custard	110 "
*Pudding, lemon	115 "
*Jam, peach	15 "
Roll, white	40 "

Total weight = 1925 gm.
Total volume = 2820 ml.

*Analyzed for vitamin C
Total weight = 770 gm.
Total volume = 1350 ml.

Low Salt Diet—(five day collection)
Jan. 28 to Feb. 1, 1946

Day 1—Jan. 28, 1946

Milk (3½%)	50 gm.
Milk, "Superior", 10%	190 "
Bread, white, salt-free (s.f.)	80 "
Butter, s.f.	25 "
Cream of wheat, s.f.	170 "
Egg, soft cooked, 1, E.P.	55 "
Soup, cream of beet	85 "
Chicken, cold, sliced	50 "
Beef, roast	40 "
*Potato, steamed	90 "
*Potato, baked	100 "
*Peas, green	65 "
*Spinach	55 "
*Peaches, canned	55 "
Custard	120 "
*Jam, peach	10 "
*Grapefruit, half	95 "

Total weight = 1335 gm.
Total volume = 2700 ml.

*Analyzed for vitamin C
Total weight = 470 gm.
Total volume = 1000 ml.

Appendix I—*Cont'd.*

Low Salt Diet, cont'd.

Day 2—Jan. 29, 1946

Milk (3¼%)	155 gm.
Milk, "Superior", 10%	65 "
Bread, white, s.f.	95 "
Butter, s.f.	30 "
Cream of wheat, s.f.	160 "
Egg, soft cooked, 1, E.P.	50 "
*Soup, cream of tomato	130 "
Beef, roast	60 "
Beef, cold, sliced	45 "
*Potato, steamed	95 "
*Potato, baked	30 "
*Carrots	60 "
*Spinach	60 "
*Orange, half	35 "
*Plums, canned	110 "
*Jam, peach	15 "

Total weight = 1285 gm.
Total volume = 2780 ml.

*Analyzed for vitamin C
Total weight = 535 gm.
Total volume = 1030 ml.

Day 3—Jan. 30, 1946

Milk, "Superior", 10%	205 gm.
Bread, white, s.f.	115 "
Butter, s.f.	30 "
Cream of wheat, s.f.	170 "
Egg, soft cooked, 1, E.P.	55 "
*Soup, cream of tomato	100 "
Chicken, cold, sliced	55 "
Turkey, cold, sliced	35 "
*Potato, steamed	145 "
*Potato, baked	75 "
Beets, diced	45 "
*Peas, green	50 "
*Cranberry sauce	20 "
*Tomato juice	75 "
*Jam, peach	20 "
Cake, chocolate with icing	110 "
Cake, apple upside down	185 "

Total weight = 1490 gm.
Total volume = 3080 ml.

*Analyzed for vitamin C
Total weight = 485 gm.
Total volume = 960 ml.

Low Salt Diet, cont'd.

Day 4, Jan. 31, 1946

Milk, "Superior", 10%	165 gm.
Bread, white, s.f.	95 "
Butter, s.f.	25 "
Cream of wheat, s.f.	200 "
Egg, soft cooked, 1, E.P.	65 "
*Soup, cream of green bean	90 "
Beef stew, plain	85 "
Beef, cold, sliced	65 "
*Potato, steamed	215 "
*Parsnips	50 "
*Tomatoes, stewed, canned	125 "
*Orange, half	30 "
Plums, canned	125 "
Pudding, bread	115 "

Total weight = 1450 gm.
Total volume = 2920 ml.

*Analyzed for vitamin C
Total weight = 510 gm.
Total volume = 950 ml.

Day 5—Feb. 1, 1946

Milk, "Superior", 10%	215 gm.
Bread, white, s.f.	135 "
Butter, s.f.	30 "
Brex	150 "
Egg, soft cooked, 1, E.P.	55 "
Egg, poached	45 "
*Soup, cream of potato	90 "
Salmon, steamed	90 "
*Potato, steamed	110 "
*Tomatoes, stewed, canned	125 "
*Peas, green	60 "
*Orange, half	40 "
Custard	75 "
Ice cream	50 "

Total weight = 1270 gm.
Total volume = 2370 ml.

*Analyzed for vitamin C
Total weight = 425 gm.
Total volume = 950 ml.

Appendix—*Cont'd.*

Low Fat Diet—(five day collection)
Jan. 28 to Feb. 1, 1946

Day 1—Jan. 28, 1946

Milk (3¼%)	260 gm.
Bread, white	70 "
Bread, brown	10 "
Butter	5 "
Cream of wheat	200 "
Chicken, cold, sliced	40 "
Beef, roast	60 "
*Potato, steamed	120 "
*Potato, baked	75 "
*Spinach, canned	80 "
*Peas, green	45 "
*Tomato juice	150 "
*Cherries, canned	90 "
*Peaches, canned	65 "
*Fruit juice, mixed	90 "
*Jam, peach	40 "

Total weight = 1400 gm.
Total volume = 2620 ml.

*Analyzed for vitamin C
Total weight = 755 gm.
Total volume = 1200 ml.

Day 2—Jan. 29, 1946

Milk (3¼%)	220 gm.
Bread, white	95 "
Butter	10 "
Cream of wheat	165 "
Beef, roast	60 "
Beef, cold, sliced	55 "
*Potato, steamed	90 "
*Potato, baked	40 "
*Carrots	60 "
*Spinach	70 "
*Tomato juice	150 "
*Soup, tomato	100 "
*Peaches, canned	90 "
*Plums, canned	90 "
*Jam, peach	50 "

Total weight = 1345 gm.
Total volume = 2790 ml.

*Analyzed for vitamin C
Total weight = 740 gm.
Total volume = 1250 ml.

Low Fat Diet, cont'd.

Day 3—Jan. 30, 1946

Milk (3¼%)	275 gm.
Bread, white	75 "
Cornflakes	25 "
Turkey, cold, sliced	40 "
Chicken, cold, sliced	45 "
*Potato, steamed	120 "
*Potato, baked	50 "
*Peas, green	45 "
Beets, diced	40 "
*Tomato juice	75 "
*Soup, tomato	100 "
*Prune whip	80 "
*Peaches, canned	95 "
*Jam, peach	50 "

Total weight = 1115 gm.
Total volume = 2300 ml.

*Analyzed for vitamin C
Total weight = 615 gm.
Total volume = 1100 ml.

Day 4—Jan. 31, 1946

Milk (3¼%)	250 gm.
Bread, white	80 "
Butter	5 "
Cream of wheat	190 "
Stew, beef, plain	100 "
Beef, cold, sliced	70 "
*Potato, steamed	220 "
*Parsnips	60 "
*Carrots	55 "
*Peach juice	50 "
*Soup, green bean	90 "
*Cherries, canned	85 "
Pudding, cornstarch, vanilla	85 "
*Jam, peach	45 "

Total weight = 1385 gm.
Total volume = 2380 ml.

*Analyzed for vitamin C
Total weight = 605 gm.
Total volume = 1050 ml.

Appendix—*Cont'd.*

Low Fat Diet, concl'd.

Day 5—Feb. 1, 1946

Milk (3½%)	405 gm.
Bread, white	115 "
Butter	5 "
Cream of wheat	150 "
Salmon, steamed	90 "
Chicken, cold, sliced	45 "
*Potato, steamed	90 "
*Potato, baked	35 "
*Peas, green	60 "
Beets, diced	30 "
*Tomato juice	105 "
*Soup, potato	100 "
*Peaches, canned	110 "
*Cherries, canned	85 "
*Jam, peach	60 "
Total weight =	1485 gm.
Total volume =	2700 ml.

*Analyzed for vitamin C

Total weight = 645 gm.
Total volume = 1120 ml.

Full Fluid Diet (five day collection) Feb. 18 to 22, 1946

Day 1—Feb. 18, 1946

Milk 3½%	640 gm.
Gruel, cream of wheat	50 "
*Soup, cream of pea	120 "
*Soup, cream of carrot and pea (strained)	135 "
*Grapefruit juice	145 "
*Tomato juice	150 "
*Peach juice	120 "
Total weight =	1360 gm.
Total volume =	1460 ml.

*Analyzed for vitamin C

Total weight = 670 gm.
Total volume = 1120 ml.

Day 2—Feb. 19, 1946

Milk (3½%)	620 gm.
Gruel, rolled oats	10 "
*Soup, cream of carrot (strained)	135 "
*Soup, cream of potato (strained)	110 "
*Tomato juice	390 "
Jello	155 "
Ice cream	50 "
Total weight =	1470 gm.
Total volume =	1900 ml.

*Analyzed for vitamin C

Total weight = 635 gm.
Total volume = 1150 ml.

Full Fluid Diet, cont'd.

Day 3—Feb. 20, 1946

Milk (3½%)	615 gm.
Gruel, rolled oats	40 "
*Soup, cream of corn (strained)	125 "
*Soup, cream of tomato (strained)	115 "
*Tomato juice	225 "
*Apple juice	120 "
Jello	120 "
Total weight =	1360 gm.
Total volume =	1460 ml.

*Analyzed for vitamin C

Total weight = 585 gm.
Total volume = 1200 ml.

Day 4—Feb. 21, 1946

Milk (3½%)	560 gm.
Gruel, cream of wheat	90 "
*Soup, cream of potato (strained)	130 "
*Soup, cream of onion (strained)	115 "
*Tomato juice	120 "
*Apple juice	125 "
*Grapefruit juice	100 "
Jello	110 "
Total weight =	1350 gm.
Total volume =	1700 ml.

*Analyzed for vitamin C

Total weight = 590 gm.
Total volume = 1100 ml.

Day 5—Feb. 22, 1946

Milk (3½%)	360 gm.
Gruel, cream of wheat	50 "
*Soup, cream of tomato (strained)	105 "
*Soup, cream of carrot (strained)	115 "
*Tomato juice	120 "
*Peach juice	130 "
*Grapefruit juice	100 "
Total weight =	980 gm.
Total volume =	1000 ml.

*Analyzed for vitamin C

Total weight = 570 gm.
Total volume = 900 ml.

Appendix I—*Concl'd.*

Clear Fluid Diet (3 day collection)
Mar. 5 to 7, 1946

Day 1—Mar. 5, 1946

Broth	215 gm.
Jello	275 "
*Tomato juice	135 "
*Apple juice	155 "
*Orange juice (fresh)	120 "
Total weight =	900 gm.
Total volume =	870 ml.

*Analyzed for vitamin C
Total weight = 410 gm.
Total volume = 750 ml.

Day 2—Mar. 6, 1946

Broth	245 gm.
Jello	230 "
*Tomato juice	265 "
*Apple juice	170 "
Total weight =	910 gm.
Total volume =	900 ml.

*Analyzed for vitamin C
Total weight = 435 gm.
Total volume = 1120 ml.

Day 3—Mar. 7, 1946

Broth	215 gm.
Jello	135 "
*Apple juice	150 "
*Tomato juice	130 "
*Grapefruit juice (fresh)	140 "
Total weight =	770 gm.
Total volume =	920 ml.

*Analyzed for vitamin C
Total weight = 420 gm.
Total volume = 930 ml.

Soft Diet (three day collection)
Mar. 5 to 7, 1946

Day 1—Mar. 5, 1946

Milk (3½%)	100 gm.
Roll, white	40 "
Butter	10 "
Cream of wheat	120 "
Egg, soft cooked, 1, E.P.	65 "
Cheese soufflé	115 "
Macaroni and cheese	175 "
*Potato, mashed	90 "
*Tomato juice	120 "
*Soup, vegetable (strained)	160 "
*Soup, creamed onion (strained)	120 "
*Applesauce	125 "
Custard	135 "
Total weight =	1375 gm.
Total volume =	2000 ml.

*Analyzed for vitamin C
Total weight = 615 gm.
Total volume = 1100 ml.

Soft Diet, cont'd.

Day 2—Mar. 6, 1946

Milk (3½%)	295 gm.
Roll, white	40 "
Bread, white	60 "
Butter	15 "
Soda crackers	20 "
Rolled oats	150 "
Egg, soft cooked, 1, E.P.	55 "
Egg, scrambled	100 "
Cottage cheese	130 "
*Soup, vegetable (strained)	120 "
*Soup, cream of corn (strained)	125 "
*Potato, mashed	105 "
*Tomato juice	130 "
*Peaches, canned	90 "
Chocolate blanc mange	120 "
Total weight =	1555 gm.
Total volume =	2100 ml.

*Analyzed for vitamin C
Total weight = 570 gm.
Total volume = 1000 ml.

Day 3—Mar. 7, 1946

Milk (3½%)	295 gm.
Roll, white	50 "
Butter	25 "
Bread, white	30 "
Soda crackers	15 "
Brex	145 "
Egg, soft cooked, 1, E.P.	60 "
Egg, creamed	155 "
*Soup, cream of vegetable (strained)	155 "
*Soup, cream of celery (strained)	110 "
*Potato, mashed	100 "
*Potato, baked	110 "
*Tomato juice	150 "
*Banana	80 "
Custard	110 "
Total weight =	1590 gm.
Total volume =	2000 ml.

*Analyzed for vitamin C
Total weight = 705 gm.
Total volume = 1150 ml.

Tube Feeding, Date of collection, 1 feeding, Feb. 23, 1946

Milk, "Superior", 10%	1077.3 gm. (38 oz.)
Milk (3½%)	907.2 gm. (32 oz.)
Sugar	1½ cups
Eggs	5
Skim milk powder	1 cup
Vitamin C tablet	1
B-plex (4 drams)	4 tsp.
Betalin tablet (1 cc.) (10 mgm.)	1
Percomorph	8 drops
Total volume =	2600 ml.

ASSESSMENT OF DIETS: ANALYSIS VERSUS COMPUTATION FROM FOOD TABLES[1]

By G. Hunter,[2] J. Kastelic,[3] and M. Ball[4]

Abstract

Analytical values for nine constituents of 12 diets are compared with corresponding values computed from food tables on the same diets. Factors tending to concordance or divergence in the values obtained by the two methods are discussed.

Introduction

The assessment of diets by direct analysis immediately before their ingestion is indisputably the best means of determining their nutritive value. Assuming accuracy in the collection of the diets, and also reliability of the analytical techniques used for measuring the food constituents—which assumptions are practicably approachable—the direct analytical method can be regarded as capable of yielding a high degree of correctness. Its limitation lies in yielding information valid only for a short period. This is partially overcome by collecting diets over a period of about a week, as has been done in the preceding paper. This week's information might be regarded as approximately valid for a month, or roughly valid for a season; many factors enter such judgments. Perhaps the most important consideration that tends to be overlooked is that an institutional or family diet that proved to be good or deficient during one week of the year is likely to be similar, under similar circumstances—especially economic—at any other time of the year.

On the other hand the assessment of diets by computation from food tables must inevitably carry uncertainties because of the great variability in the composition of natural foods and the greater variability brought about by modern methods of food processing. No amount of analysis and no statistical treatment of the results can overcome the uncertainty of choice of a basic value to compute a constituent of any particular diet.

However, despite the theoretical shortcomings of the best computation methods the fact remains that they have a large measure of usefulness. There is no question that competent dietitians can readily assess diets approximately for most constituents of interest in nutrition today. The extensive literature on food analysis, summarized in food tables, makes this possible. The effects of seasonal variation, storage, cooking, and various other factors can be

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[4] *Technician.*

approximated by experienced computers, and practically the whole of the literature on dietary surveys is dependent on this method—applied with greatly varying amounts of observational data.

There has been, however, a perceptible tendency to approach the more direct analytical method. A recent paper by Kaser *et al.* (7) compares "calculated and determined calorie and vitamin contents of mixed diets". The investigators here collected three-day dietary intake records from a population of about 1100 people of all ages. "From these records 80 representative diets for 1 day were selected for duplication, 40 each from the fall and spring periods". The diets were all prepared and cooked under the supervision of the investigators.

Largely because of the rather elaborate organization required for direct analysis there are few recorded comparisons between analytical and computed values. Thus when planning the study reported in the previous paper it was decided to collect the data necessary for subsequent computation of values from food tables, as a test of the computation method.

Methods

A list was made of the weight of every food item for each day for all the diets listed in the appendix to the preceding paper.*

A list was then made of all food items with columns of food table values of the items studied, and containing under each food item the amount present each day in each diet.

From this list the total protein, calcium, thiamine, etc. were summed for each diet over the period studied. All sums were divided by the number of days the diets were collected, so that all results are finally expressed on an average daily basis.

References 1–5 and 9–12 show the food tables used.

In the use of the food tables we endeavored to choose the basic value for the computing of each constituent of each food item as that most likely to apply to our conditions. As far as possible allowance was made for cooking, seasonal variation in food composition, and like factors.

In a few instances, where probable values were not obtainable from the literature, we used our own analytical values, and a few data from unpublished tables used in a previous nutritional survey (6).

Results

In Table I are assembled the values as found by analysis alongside the corresponding values found by computation from food tables.

There are appended to Table I calculated values for carbohydrate, fat, and calories in the diets, with sedentary values for men and women as recommended by the Food and Nutrition Board, National Research Council, U.S.A. (8).

* *Hunter, G., Kastelic, J., and Ball, M. Assessment of hospital diets. Can. J. Research, E, 26 : 347–366. 1948.*

TABLE I

HOSPITAL DIETS: DAILY CONSTITUENT INTAKE. VALUES OBTAINED BY ANALYSIS COMPARED WITH VALUES COMPUTED FROM FOOD TABLES

Diet	Vit. A + carotene, I. U.		Ascorbic, mgm.		Thiamine, mgm.		Riboflavin, mgm.		Niacin, mgm.		Calcium, gm.		Phosphorus, gm.		Iron, gm.		Protein, gm.		CHO, gm.	Fat, gm.	Calories
	Analysis	Computed	Analysis	Computed	Analysis	Computed	Analysis	Computed	Analysis	Computed	Analysis	Computed	Analysis	Computed	Analysis	Computed	Analysis	Computed	Computed		
Full House	9700	9187	25	54	0.43	1.04	1.08	1.96	5.95	9.72	1.29	0.89	1.66	1.49	12.46	13.12	80.3	75.6	239.6	74.0	1900
Progressive Sippy									0												
1 to 5 days	1700	3910	3	2	0.40	0.36	2.06	1.55	0.76	0.95	—	1.06	1.24	0.91	1.76	2.21	35.5	34.7	46.5	96.1	1200
6th day	2700	5495	—	2	0.21	0.58	1.38	2.14	0.76	1.05	—	1.14	1.68	1.25	6.12	6.53	45.4	55.2	47.6	114.5	1400
7 to 8 days	3200	5060	1	2	0.40	0.52	1.72	1.98	0.75	1.02	1.04	1.13	1.60	1.17	5.45	6.05	43.2	53.9	77.1	109.4	1400
9 to 10 days	3700	5340	2	2	0.44	0.58	2.48	2.12	0.82	1.12	1.05	1.23	1.64	1.31	4.67	7.39	53.0	62.6	116.3	114.7	1500
11 to 12 days	1000	5145	4	2	0.53	0.68	1.68	2.15	0.82	1.42	1.06	1.27	1.76	1.32	3.17	5.91	53.1	60.3	117.5	117.0	1700
13 to 15 days	1600	4815	9	15	0.74	0.74	1.66	2.03	1.83	2.50	0.96	1.19	1.91	1.23	5.15	5.97	55.7	58.4	153.5	114.0	1900
16 to 21 days	2000	6060	11	11	0.76	0.93	2.09	2.53	6.78	3.51	1.14	1.34	2.09	1.45	4.37	7.98	58.1	70.9	180.9	144.8	2300
Initial Postulcer	12,400	8074	29	60	0.97	1.27	1.57	3.07	8.17	6.41	1.57	1.61	2.63	1.88	8.77	11.50	100.7	97.6	259.4	129.5	2700
Admission Diabetic																					
(Adult's)	7600	6132	90	110	0.44	0.99	1.22	1.44	9.65	11.75	0.49	0.77	1.35	1.40	11.90	13.15	80.8	74.1	217.7	70.7	1500
(Child's)	4800	8579	56	86	0.62	0.85	1.29	1.81	7.23	6.08	0.89	1.10	1.00	1.28	9.12	10.24	56.9	52.0	166.4	67.1	1300
Low Residue	6400	5254	20	69	0.65	1.00	2.01	2.16	8.75	6.27	1.02	1.11	1.88	1.37	7.23	10.05	86.8	80.8	221.0	83.5	2100
Low Salt	6600	6844	34	61	0.80	0.82	1.25	1.22	9.45	10.15	0.71	0.58	1.20	0.97	13.30	11.93	82.3	70.0	228.4	68.0	1800
Low Fat	5800	7578	21	65	0.61	0.76	1.10	1.16	3.72	10.64	0.68	0.69	1.06	0.93	6.69	11.35	74.2	62.9	229.2	28.5	1400
Full Fluid	3800	1796	58	87	0.24	0.55	1.31	1.39	3.76	0.72	0.89	0.73	0.77	0.63	5.56	3.25	30.7	27.6	97.8	23.0	800
Clear Fluid	2200	632	118	70	—	0.30	1.70	0.35	5.05	0.25	0.11	0.18	0.20	0.21	13.45	2.00	17.6	10.3	90.8	6.1	400
Soft Diet	3000	3712	15	47	0.20	0.95	1.55	1.87	2.90	3.71	0.96	0.74	1.46	1.17	7.12	10.34	70.3	67.1	173.2	69.9	1600
Tube Feeding	18,200	16,500	2	120	11.31	11.24	9.10	9.70	22.88	21.05	3.12	3.73	2.11	3.21	6.50	8.90	119.3	130.8	464.0	170.0	3900
Men, sedentary	5000		75		1.2		1.6		12		0.8				12.0		70				2500
Women, sedentary	5000		70		1.2		1.5		12		0.8				12.0		60				2100

Discussion

On comparing analytical and computed values for the different constituents a greater measure of concordance is found than might have been expected. Adequacy or deficiency is in general made plain by either value. Both methods show clearly that in nearly all diets there is more than adequacy of calcium and phosphorus and less than adequacy of iron, ascorbic acid, and thiamine. Such general concordance is evidence for the general trustworthiness of the computation method based on accurate food amounts.

The values for iron by the two methods are surprisingly concordant with the exception of that of the Clear Fluid diet. The juice of green vegetables which escaped our computation method was responsible for the relatively high iron value found by analysis.

The greatest divergence between analytical and computed values is found in the vitamins. The vitamin A by analysis in the Sippy diets is rarely half, and in one case only one-fifth of the computed values. It appears that the computation factors chosen for milk (170 I.U. per 100 ml.), for cream (400 I.U. per 100 ml.), and perhaps also that for eggs, were too high for these Edmonton foods for the month of October.

It is of interest to note that in the more varied diets the analytical and computed values for vitamin A are fairly concordant: and most likely not because our factors for other items were more accurate but because in a varied diet errors in the choice of basic computing factors have a tendency to cancel each other. As a corollary to this we see that in the Sippy diet an error in choice of computing factor throws the values all out in one direction; e.g. computed vitamin A values are all too high, computed phosphorus all too low—but in the varied diets the divergence for the one constituent is likely to swing in either direction—too high or too low. By induction we might state it as a general principle that the greater the number of different food items in a diet the greater the chances that the computed constituent values will be correct.

It is obvious that such a rule will not apply to an unstable constituent like ascorbic acid if analysis is not carried out promptly or adequate means adopted to prevent oxidation before analysis. In such a case the computing factor used is likely to be so erroneously high that constituent values for all diets will analyze consistently low.

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